Animal trypanosomiasis in the Eastern Province of Zambia: epidemiology in the recently-settled areas and evaluation of a novel method for control

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Declaration

I, Joseph Mubanga, do declare that the research described in this thesis is my own work and that it has not been submitted for any other degree or professional qualification.

Signed

Acknowledgment

My special thanks go to Dr Mark C. Eisler and Professor Susan C. Welburn, my supervisors for the guidance they provided to me throughout these studies, including my field research in Zambia, PCR analysis at the Centre for Tropical Veterinary Medicine (CTVM) Laboratories and compilation of this thesis. I am also grateful to Dr. Kim Picozzi at CTVM for her advice in the laboratory when I was doing the PCR analysis for trypanosomiasis and theileriosis. The kind co-operation of Dr. Andrew Brownlow in the restricted application of insecticide on cattle to control tsetse- and tick-borne diseases in Petauke District, Zambia is gratefully acknowledged.

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Contents

List of tables

List of figures

List of abbreviations

AAT	African Animal Trypanosomiasis
Ab-ELISA	Antibody-detection ELISA
ADB	Africa Development Bank
Ag-ELISA	Antigene-detection ELISA
ANOVA	Analysis of Variance
ASVEZA	Assistance to Veterinary Services of Zambia
BADCP	Belgium Animal Disease Control Project
BCT	Buffy Coat Technique
CATT	Card Agglutination Trypanosomiasis Test
CFT	Complement Fixation Test
CI	Confidence Interval
CNS	Central Nervous System
CTVM	Centre for Tropical Veterinary Medicine
CVRI	Central Veterinary Research Institute
DACO	District Agriculture Co-ordinator
DFID	Department for International Development funds
dl	Decilitre
DNA	Deoxyribonucleic Acid
DVLD	Department of Veterinary and Livestock Development
EBVC	Easter Bush Veterinary Centre
ECF	East Coast Fever
EDTA	ethylenediaminetetraacetic acid
EEC	European Economic Community
ELISA	Enzyme-Linked Immunobsorbent Assay.
EU	European Union
FAO	Food and Agriculture Organisation
FTA	Free to Air
g/dl	Grams per decilitre
GDP	Gross Domestic Product
GIS	Geographical Information System
GMA	Game Management Area
GMA	Game Management Area

GMBH	Gesellschaft mit beschränkter Haftung
GPS	Global Positioning System
HAT	Human African Trypanosomiasis
Hb	Haemoglobin
HCT	Haematocrite Centrifugation Technique
IFAT	Indirect Fluorescent antibody Test
ILRAD	International Laboratory for Research on Animal Diseases
ILRI	International Livestock Research Institute
ISSM	Isometamidium Chloride
ITS	Internal Transcribed Spacers
KCI	Potassium Chloride
Kg	Kilogram
$MgCl_2$	Magnesium Chloride
ml	Millilitre
mm	Millimetre
NP	National Parks
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
RTTCP	Regional Tsetse and Trypanosomiasis Control Programme
RTTCP	Regional Tsetse and Trypanosomiasis Control Programme
SIT	Sterile Insect Technique
Spp	Species
SRA	Serum Resistance Associated gene
StDev	Standard Deviation
T&T	Thick and thin
Tb	*Trypanosma brucei*
Tc	*Trypanosoma congolense*
Tv	*Trypanosoma vivax*
UK	United Kingdom
US$	United States Dollar
USA	United States of America
VA	Veterinary Assistant
VO	Veterinary Officer

WHO	World Health Organisation
ZAWA	Zambia Wildlife Authority
ZMK	Zambian Kwacha
µL	MicroLitre

Abstract

The Eastern Province of Zambia includes a range of ecosystems between the Luangwa river valley at its north-western boundary and the Eastern plateau which extends to its southern boundary, resulting in clines in the distributions of the tsetse and tick species that are important vectors of trypanosomiasis and a number of tick-borne diseases of domestic livestock. Factors affecting the epidemiology and impact of trypanosomiasis in livestock were studied in households in Mambwe District in an area extending from the Luangwa river valley to the edge of the eastern plateau. A structured questionnaire survey on demography, migration and farm activities of householders showed that about 84% of the households depended on farming for their income, mainly cotton growing. People living in this area are poor with average income per household per annum of about K1,849,131 (US$377.85). Approximately 50% of householders were immigrants to the district, and of these 80% arrived in the last ten years. A cross-sectional study of 649 cattle, 811 goats, 58 sheep and 177 pigs in these households used Giemsa-stained blood smears and ITS-PCR amplification for diagnosis of trypanosomiasis. Prevalence was highest in cattle (28.4% [95%CI: 23.7-33.5]) followed by pigs (21.5% [95%CI: 13.9-31.8]), sheep (18.2% [95%CI: 5.1-47.7]) and goats (9.2% [95%CI: 6.8-12.4]). The prevalence within households depended on the particular combinations of livestock species kept; small ruminants were more likely to be infected if cattle were also present. In cattle, prevalence ranged from 26.3% (95%CI: 19.6-34.2) above 700m above sea level to 44.1% (95%CI: 36.9-51.6) below 600m. *Trypanosoma congolense* (savannah type) was identified in 82.4% of all trypanosome-infected cattle, *T. vivax* in 24.5%, *T. brucei* in 2.7%. *Trypanosoma simiae* was only identified in pigs (27.8% of infected pigs).

Finally, 'restricted application', a novel modality of use of synthetic pyrethroid to control both tsetse- and tick-borne diseases of cattle was investigated in a longitudinal intervention study in 12 villages in Petauke District on the eastern plateau. Baseline data showed that trypanosomiasis was more prevalent in villages in the northern part of the study area while theileriosis was more prevalent in southern villages. Existing infection of trypanosomes were treated with two doses of diminazene aceturate 42 and 14 days prior to interventions. Spraying only the limbs,

belly (predilection feeding sites of tsetse) and ears (predilection feeding site of *Rhipicephalus* species ticks) of cattle with dilute deltamethrin, was compared with conventional pour-on application of deltamethrin, trypanocidal chemoprophylaxis using isometamidium chloride, and non-intervention controls. Each intervention (or non-intervention) was applied to 80 cattle in each of 3 villages, monthly (deltamethrin) or just once (isometamidium). The subsequent incidence of trypanosome infection was too low to make a meaningful conclusion on the interventions. Nevertheless restricted application had significant effect on tick infestations and animals treated with deltamethrin showed lower cumulative incidence of *Theileria* species.

CHAPTER ONE

1. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. The importance of keeping livestock in the sub-Saharan African countries

For rural populations in sub-Saharan Africa livestock are an important source of income, in the form of milk, meat, hides and draught power services. Livestock also serve as a form of saving or investment and in times of need may be sold to generate cash to pay for things like school and medical fees. Livestock provide draught power for crop cultivation, manure for maintaining soil fertility and milk and meat for household consumption (Perry *et al.*, 2002). In 1988, agriculture contributed 32% of the gross domestic product to the sub-Saharan region, and livestock products represented 35% of agricultural domestic product (Odongo, 2004)

In Zambia, agriculture is critical for poverty reduction and the economic development of the country. This sector generates between 18 to 20% of the Gross Domestic Product (GDP) and the majority of rural people (more than 60% of the population) derive their livelihood from agricultural related activities (Zambia, Fifth National Development plan 2006-2010, 2005). Livestock are a crucial part of the agriculture sector providing food products, employment and income. Through draught power the livestock sub-sector contributes directly to increased agricultural production in general and food security in particular accounting for about 35% of the total agricultural production (Lungu, 2003). The main livestock species reared in Zambia are cattle (2,341,970), goats (1,002,376), pigs (286,726), sheep (97,605) and poultry (DVLD, Annual report, 2004).

1.2. The effect of tsetse-borne and tick-borne diseases on livestock production and livelihoods in the sub-Saharan Africa.

It is generally recognised that disease is the greatest constraint to animal health and production in sub-Saharan Africa (Perry *et al.*, 2002). Tsetse and tick-borne diseases cost Africa around US$4-5 billion per year in livestock production-associated losses. Approximately 80% of the world's cattle population are at risk from ticks and tick-

borne diseases (Minjauw *et al.*, 2003). World wide the cost associated with ticks and tick-borne diseases in cattle is estimated to be between US$13.9 billion and US$18.7 billion (de Castro, 1997).

Trypanosomiasis is considered the most important infectious disease holding back the development of livestock production in much of sub-Saharan Africa (ILRAD reports, April 1993). It has been estimated that 48 million cattle (Kristjanson *et al*, 1999) are at risk of contracting trypanosomiasis. The disease has directly contributed to the widespread malnutrition in Africa by decreasing the amount of protein food available to man (Mulligan 1970). Trypanosomiasis transmitted by the tsetse flies has been estimated to cost Africa US$4.5 billion a year (Eisler *et al.*, 2003). It has been estimated that the annual income losses (GDP) for the 10 African countries completely infested by tsetse is in the range of US$192 to US$960 million (Swallow, 2000). If tsetse flies are controlled, it is estimated that there will be about 16% and 18% increase in meat and milk production in sub-Saharan Africa (Tacher *et al.*, 1987, Thuranira, C.M., 2005). This disease costs the producers and governments an estimated US$35 million per year assuming treatment per animal is US$1 (Kristjanson *et al.*, 1999). The disease reduces cattle population density by up to 30% to 50% and the meat and milk off take by about 50% in addition to reducing the calving rate by 20% of infected animals (Swallow, 2000).

In much of Zambia, bovine trypanosomiasis retards agriculture development as it is impractical to keep domestic animals in areas heavily infested with tsetse flies (Van den Bossche, 1997). Trypanosomiasis continues to be a major cause of economic losses in the country, especially in the Eastern, Northern, Lusaka, Southern and Western provinces (Figure 1.1). However, theileriosis a tick-borne disease accounts for the largest number of cattle deaths in the country and is more prevalent in Eastern and Southern provinces (DVLD, Annual reports. 1919 -2004).

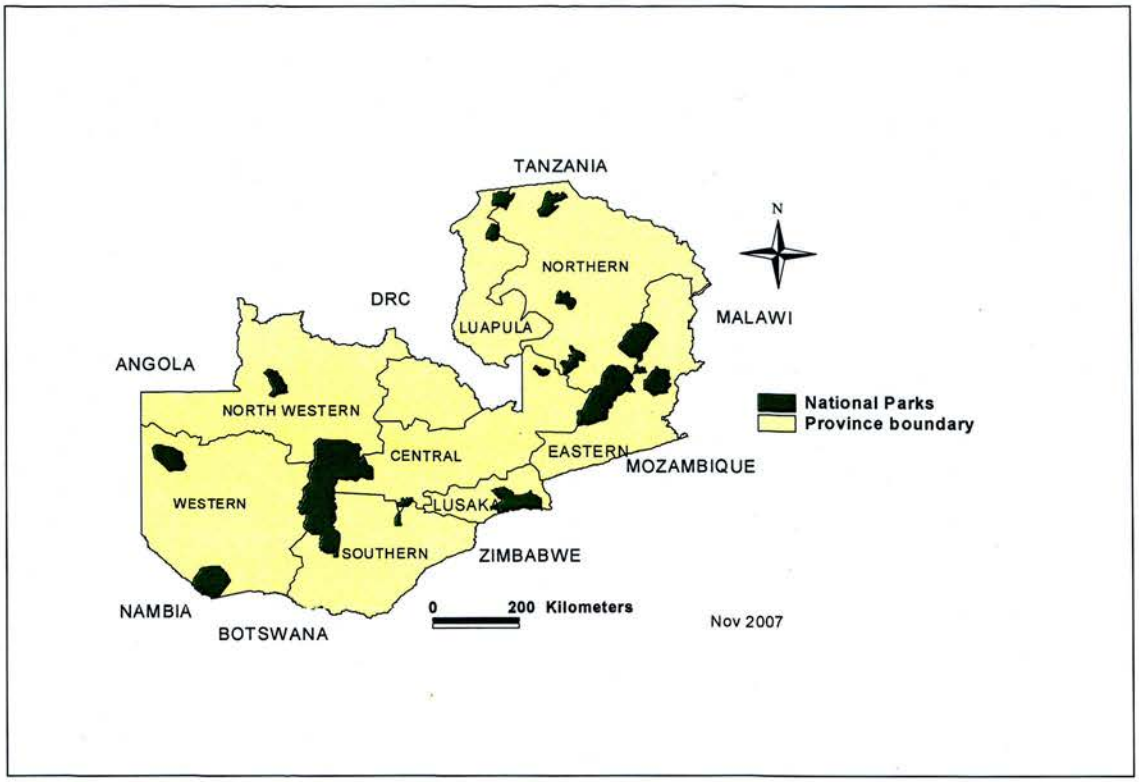


Figure 1.1 Map of Zambia showing the nine provinces.

1.3. The importance of epidemiology of tsetse-borne and tick-borne diseases

Information on the distribution and feeding behaviour of the arthropod vectors of disease is essential in understanding the relationship between hosts and vectors, and their respective roles in a disease transmission cycle (Tempelis, 1975). In the tsetse fly belt of sub-Saharan Africa, trypanosomes the parasites that cause trypanosomiasis are mainly transmitted cyclically by their vector the tsetse fly (*Glossina* spp., Diptera: Glossinidae). The epidemiology of tsetse transmitted trypanosomiasis is mainly determined by tsetse density or challenge, infection rate and host preference (Connor, 1993; Clausen, *et al*, 1998).

The major tick-borne diseases are transmitted by ticks of the family Ixodidae (Hard ticks). The transmission of East Coast Fever is transstadial, with larva and nymphs picking up the infection and transmitting it in their next stage of development as nymphs and adults, respectively (Mulumba *et al.*, 2001).

1.4. Trypanosomiases

1.4.1. The parasites

1.4.1.1. Classification of trypanosomes

Trypanosomes are protozoan parasites which are members of the Order Kinetoplastida, Family Trypanosomatidae and Genus *Trypanosoma*. Based on the development site in the tsetse fly, the genus is sub-divided into two sections, the Stercoraria and Salivaria (Hoare, 1972).

Stercoraria trypanosomes develop (from epimastigotes to infective metatrypanosomes) in the hid-gut of the vector following ingestion of infected blood meal and transmitted to the mammalian host by faecal contamination (e.g. *T. theileri*). Salivaria trypanosomes (*T. vivax, T. congolense, T. brucei*) develop in the mouth parts of the vector and are transmitted to the host by inoculation when the fly is having a blood meal (Stevens and Brisse, 2004, Leach and Roberts, 1981). The species *T. congolense* is further divided into four sub-species *T. congolense (*Kilifi), *T. congolense* (Savo), *T. congolense (*savannah) and *T. congolense* (forest).

The genus is further divided in sub-genera; *Duttonella* (*T. vivax*), Nannomonas (*T. congolense, T. simiae, T. godfreyi*), Trypanozoon (*T.b.brucei, T.b.rhodesiense, T.b. gambiense*), Pycnomona (*T. suis*) and Crocodile parasite (*T. grayi*), (Stevens and Brisse, 2004)

Losos and Ikede (1972) divided the tsetse transmitted trypanosomes into two groups based on the distribution in the host. These two groups are;

i) Haematic group: these confine themselves to the blood and lymphatic systems. These include *T. vivax* and *T. congolense*.

ii) Humoral group: those that also invade connective tissue and body cavities. This group include *T. brucei* species.

1.4.1.2. General Structure of trypanosomes

The parasite is a single cell (unicellular) organism varying in size from 8-39μm (Mulligan, 1970). Morphologically species of trypanosomes differ slightly from each other in their appearance (Table 1.1). The structure in Figures 1.2 and 1.3 show the

general structure and features of the trypanosome. The features that are clearly seen in stained blood smears under the microscope and are used to differentiate the trypanosomes from each other include; surface coat, cell membrane (pellicle or cell envelope), undulating membrane, flagellum, nucleus, kinetoplast and cytoplasm. The surface coat is used as a defence mechanism against the host immunity system (Cross, G.A.M, 1975 and 1996). The undulating membrane and flagellum are used for locomotion or movement. The nucleus is the control centre of the cell and plays a major part in reproduction. The kinetoplast is where the flagellum originates from (Boyt, 1986).

Table 1.1 Morphological structure of pathogenic trypanosomes (adopted from Mulligan , 1970 with some modification).

Species	Size	Flagellum	Undulating membrane	Shape of posterial end	Size of Kinetoplast	Position of Kinetoplast
T. brucei (Polymorphic)	11-39	Long and free	Well developed	Pointed	Small	Sub-terminal
T. congolense (Monomorphic)	8-24	No free flagellum	Poor developed	round	Medium	Marginal and sub-terminal
T. vivax (Monomorphic)	18-31	Free flagellum	Slightly developed	Swollen and bluntly pointed	Large	Terminal
T. simiae (Polymorphic)	12-24	Some with Free flagellum	Well developed	round	Medium	Marginal and sub-terminal

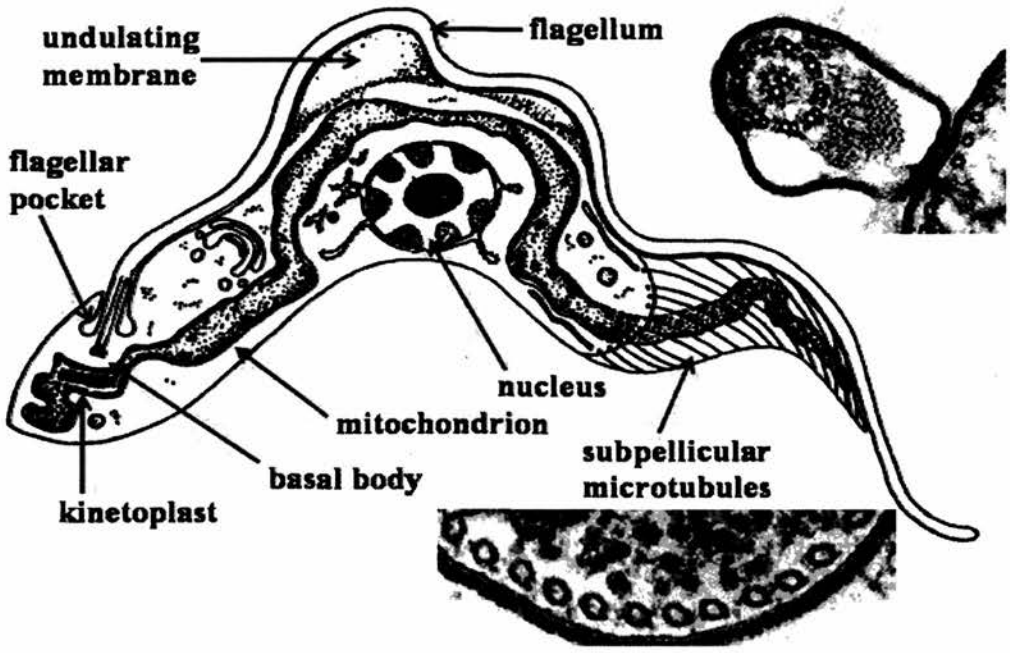


Figure 1.2General structure of a trypanosomes.

Source:www.uta.edu/biology/badon/classnotes/3444/Lecture5.htm

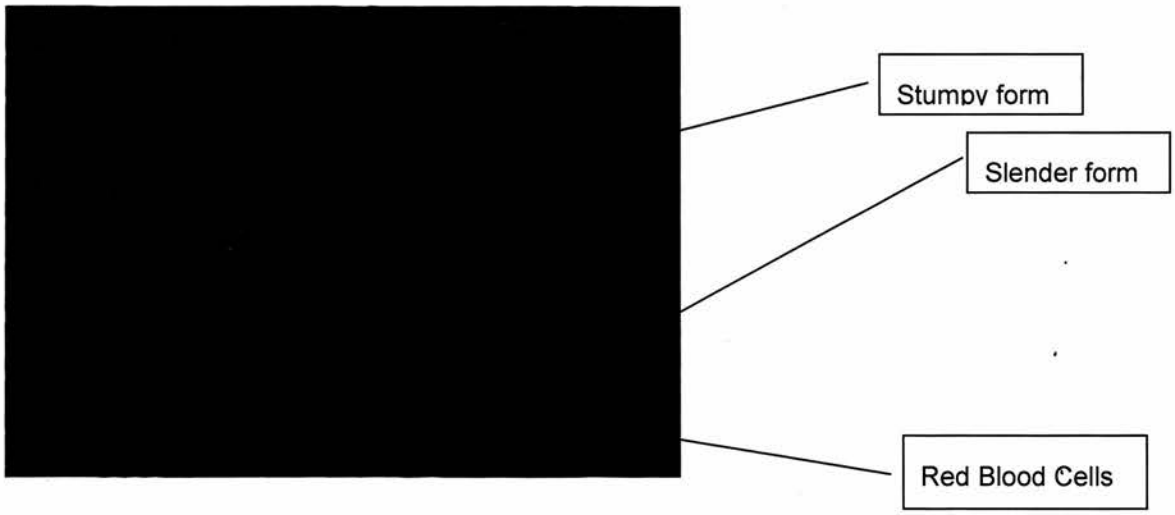


Figure 1.3 *Trypanosoma bruei* in the blood stream.

1.4.1.3. Life cycle of trypanosomes

Pathogenic Salivarian trypanosomes undergo a cyclical development in both the vector and animal or human hosts. The complex cycle involves a series of cellular transformation of the trypanosomes into different forms. In the tsetse fly, *T. vivax* development takes about 5 days while that of *T. congolense* takes 2 – 3 weeks and *T. brucei* 3 – 5 weeks (Vickerman, 1988). Non-infected tsetse flies ingest the trypomastigotes (blood form) from the animal or human hosts. In the tsetse gut trypamastigotes multiply and differentiate to the procyclic forms. These procyclic forms then move to the salivary glands (only *T. brucei*) multiply and differentiate into epimastigote. The epimatigote will later on also differentiate into metacyclic forms and it is this form that is injected into the skin of a mammalian or animal host (Figure 1.4).

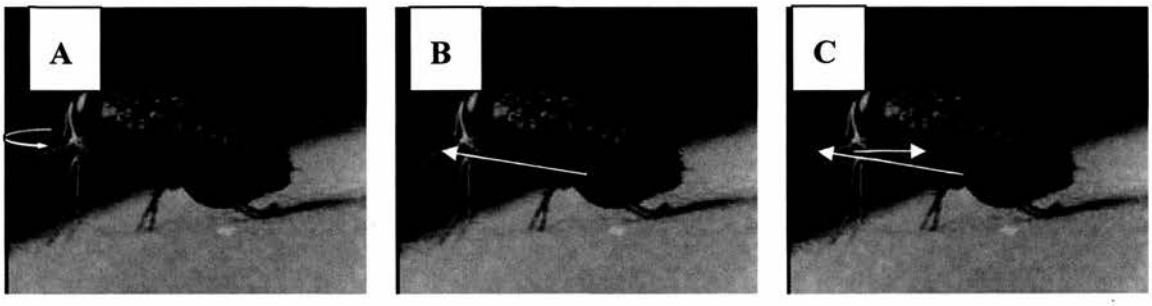


Figure 1.4 Diagram showing the sites in the tsetse fly where trypanosmes development takes place. A) *T. vivax* develops within proboscis. B) *T. congolense* develops first in the midgut and then in the proboscis. C) *T. brucei* develop first in midgut then in proboscis and finally in the salivary glands.

1.4.1.4. Species found in sub-Saharan Africa

African tsetse-transmitted trypanosomiasis is caused by protozoan parasites of the Genus *Trypanosoma*. Trypanosomiasis affecting livestock in Africa is mainly caused by *Trypanosoma congolense*, *T. brucei brucei* and *T. vivax*. Other species found in suids are *T. simiae and T. suis.* In human beings there are two species namely *T. b. rhodesiense* and *T. b. gambiense (*Hoare, 1966*).*

The major non-pathogenic species that are transmitted by tsetse flies are *T. theileri* and *T. ingens*. Others include: *T. lewisi* and *T. musculi* in rodents; *T. vespertilionis* and *T. megadermae* in bats (Hoare, 1970).

The other non-tsetse transmitted trypanosomes include; *Trypanosoma evansi* found in camel. It is transmitted mechanically by biting Diptera. *Trypanosoma equiperdum* found in horses is transmitted through coitus.

1.4.2. The Host

Trypanosomes parasitize both domestic and wild vertebrate and invertebrates hosts. In the sub-Saharan Africa the main invertebrate host parasitized are tsetse flies and tabanides (Desquesnes and Dia, 2003). These invertebrate hosts also act as trypanosome vector and transmit these parasites to human and animal hosts. The vertebrates infected with trypanosomes include mammals, fishes, amphibian (Connor, 1989) reptiles (Lefrançois, *et al.*, 1998, Nyagu, *et al.* 1999 and Jordan, 1961) and avian (Okiwelu, 1977 and Sehgal *et al.*, 2001).

Hosts that get infected but do not develop a disease are called reservoir hosts (Heisch *et al.*, 1958 and Onyango. *et al*, 1969). Wildlife, cattle, sheep, pig and goats can get infected with *T. b. rhodesiense* and *T. b. gambiense* but do not become sick (Welburn, *et al.*, 2001 and Ng'ayo, *et al.*, 2005) but only harbour these human-infective trypanosomes. Most wildlife animals can be infected with trypanosomiasis but do not develop a disease. They act as reservoir host for the trypanosomes that infect both man and his domestic animals (Woo *et al.*, 1970).

1.4.3. The disease

1.4.3.1. African Animal Trypanosomiases (AAT)

The trypanosomiases represent a disease complex both in man and in animals. In human beings it is called sleeping sickness and in animals it is generally known by three names;

i) Nagana in the sub-Saharan Africa

ii) Surra, caused by *T. evansi* in North Africa and Asia

iii) Dourine, caused by *T. equiperdum* in horses

Wild animals and the trypanosomes have evolved together for many years and so are said to have a balanced relationship (Connor, 1989). These species of animals acquire an infection but do not develop symptoms of the disease thereby allowing them to carry the disease as reservoirs of the parasites. In domestic animals the host/parasite relationship has not fully developed because they are relatively newcomers to the tsetse infested areas. Among domesticated animals, humpless cattle (N'dama of West Africa) of *Bos taurus* type tolerate trypanosomes without showing signs of the disease as long as they are not stressed by factors such as poor nutrition, working, lactation, parturition or other disease(Murry *et al,* 1982).

Some mammals possess natural resistance to particular species of trypanosomes. Cattle are not likely to be infected with *T. simiae* (Roberts, 1971)

1.4.3.1.1. Domestic animal trypanosomiasis in Zambia

Trypanosomiasis is a major cause of livestock morbidity in Zambia, especially in the area where cattle are kept in the Game Management Areas (GMAs) or in close proximity to the National Parks. In the central, western and southern part of Zambia, cases are mainly reported in areas that lie in close proximity to the Kafue National Park. In the Northern and Eastern provinces cases are reported mainly from areas near the Luangwa National Park in the Luangwa valley that is part of the Great Rift Valley of Eastern Africa. This area has been a historical focus of sleeping sickness from the time the wildlife population recovered from the Rinderpest epidemic in the early 1900s (Foulkes, J. 1970, Buyst, H. 1974). Number of cattle cases and deaths that were reported in the country between the year 1994 and 2004 are indicted in Table 1.2. Few cases were reported in the year 2000 due to inactivity of the Tsetse Control Section. This was a period when most of the donor funded tsetse control projects were phasing out. Most of the work was done in Eastern Province of Zambia. Eastern province is important because it is an area with high agriculture potential with many livestock. Zambia has a cattle population of 2,341,970 cattle out

of which 190,671 are in the Eastern Province of Zambia (DVLD, Annual Report, 2004).

Table 1.2 Data of domestic animal trypanosomiasis in Zambia.

Year	Cases	Deaths
1994	21,228	155
1995	21,241	154
1996	21,290	155
1997	15,560	253
2000	802	70
2001	2,277	280
2002	1,883	89
2004	2,985	479
Total	**87,266**	**1,635**

Source: Department of Veterinary and Livestock Development annual reports.

Thick and thin smear and buffy coat techniques were used for examinations for the above results.

Eastern Province is basically divided into two areas i.e. the plateau and valley. Tsetse density is very high in the valley and low on the plateau. As a result of this most of the cattle are kept on the plateau and most of the veterinary department livestock activities are carried out on this part of the province. Many different tsetse control projects have been done on the plateau area of the Eastern province. The Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) under the European Union (EU) funding facilities which operated in the area between the year 1990 and 2000 conducted trypanosomiasis surveys. These surveys showed that the prevalence on the plateau ranged between 0% in Chadiza and 25% in Petauke. Petauke had high prevalence because it lies near the South Luangwa National Park the source of tsetse flies. A few surveys that were conducted in the valley revealed trypanosomiasis

prevalence in the range of 16% to 24%. In the valley the true prevalence can be masked because animals are continuously on trypanocidal drugs. The results of these general surveys are tabulated below in Tables 1.3 and 1.4.

Table 1.3 Table of domestic animal trypanosomiasis on the plateau in Eastern Province.

Year of survey	Districts	Animals sampled	Animals positive	Prevalence (%)
1993	Petauke/Katete	734	188	10.20
1995	Katete	1211	114	9.5
	Petauke	2225	555	24.9
1996	Petauke/Katete	2007	214	8.53
1997	Katete	243	20	8.2
	Petauke	868	206	23.7
	Lundazi	486	4	0.82
2001	Msanzara	1008	36	3.58
	Petauke	106	5	4.72
	Chipata	118	1	0.85
2002	Chipata	520	5	0.96
	Nyimba/Petauke	428	23	5.4
	Chadiza	100	0	0

Source: RTTCP, Tsetse & Trypanosomiasis Control Section, Chilanga

Note: Diagnostic technique used was thick and thin smear of blood.

Table 1.4 Data of domestic animal trypanosomiasis for the valley in Eastern Province.

Year	Animals sampled	Animals positive	Prevalence (%)	PCV (%)
2001 Msoro	84	20	23.8	-
2002	669	112	16.75	28.57

Source: RTTCP, Tsetse & Trypanosomiasis Control Section, Chilanga

Note: Diagnostic technique used was thick and thin smear of blood.

1.4.3.2. Human African Trypanosomiasis

Human African trypanosomiases (HAT) are commonly known as sleeping sickness. There are two forms of African sleeping sickness caused by two different parasites. *Trypanosoma brucei gambiense* which causes a chronic infection and affects countries in western and central Africa. *Trypanosoma brucei rhodesiense* which causes acute illness in countries of eastern and southern Africa. The disease progresses in two stages i.e. the first is the early (haemolymphatic stage) that starts 1-3 weeks after an infective tsetse bite and last for about 1-7 days (Kennedy, 2004). The symptoms during this stage include fever, malaise, anaemia, headache, pyrexia, weight loss and weakness (Kennedy, 2004). The second stage is the late (meningoencephalitic) stage when the parasite invades the central nervous system. The symptoms include psychiatric, motor and sensory disorders, sleeping abnormalities, seizures, and coma. The disease is fatal if remains untreated (Sternberg, 2004). It has been estimated that about 50 million people (Kuzoe, 1991) are at risk of contracting trypanosomiasis. About 300,000 – 500,000 people are infected and 100,000 deaths are caused each year by the disease (Cattand *et al.*, 2001, WHO, 2001; Welburn *et al.*, 2004).

In Zambia sleeping sickness is mainly found in Luangwa valley in the Eastern and Northern provinces of Zambia (Foulkes, J. 1970, Buyst, H. 1974, Rickman, 1974).

The first documented case of sleeping sickness in Zambia was that of W. Armstrong who was diagnosed positive in 1910 came from the Luangwa valley (Buyst, 1977). In Zambia, areas in which the disease is endemic and where cases occur sporadically are quite widespread. There is a suspicion that many more cases currently remain undetected (Van Nieuwenhove, 2003). The disease is now rare in the country and can be missed or dismissed as retroviral disease, particularly in adults (Ngoma, 2004). Data of cases for human sleeping sickness from the year 1999 to 2005 for the whole country is tabulated below (Table 1.5) and for Eastern province only (Table1.6). The number of human cases in the whole country between the year 1925 and 2004 are shown in Figure 1.5 and Appendix 1. Data for the period between 1986 and 1998 was not available, so is not shown.

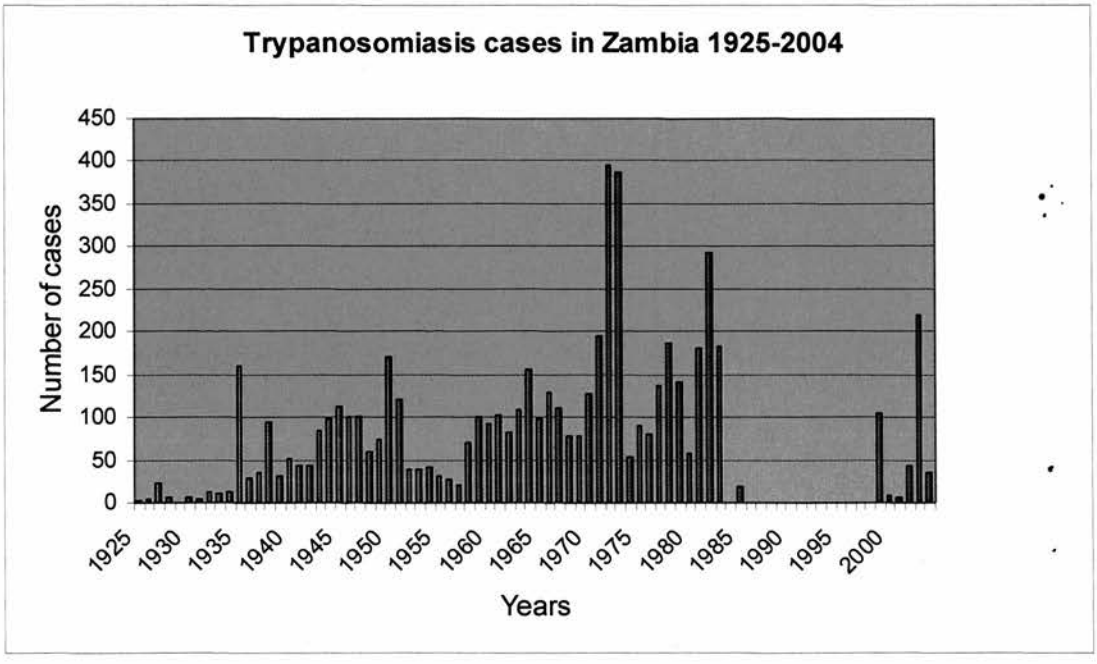


Figure 1.5 Number of human trypanosomiasis cases in Zambia from 1925 to 2004

Table 1.5 Data of human trypanosomiasis for Zambia for the year 1999 - 2005

New Cases	1999	2000	2001	2002	2003	2004	2005
Under 5 years	96	3	2	17	103	13	56
Over 5 years	8	6	4	26	116	22	45
Total:	104	9	6	43	219	35	101

Source: Ministry of Health database, Zambia

Table 1.6 Data of human trypanosomiais for Eastern Province, Zambia.

	1975	1976	1977	1978	1979	1980	1999	2002	2003
Under 5 years							0	0	1
Over 5 years							8	5	4
Total:	38	31	128	57	79	31	8	5	5

Source: WHO, Lusaka

1.4.4. The vector

1.4.4.1. Distribution of tsetse flies

Tsetse flies are only found in sub-Saharan African where they are vectors of trypanosomes, the parasites that cause trypanosomiases in both human and animals. It is estimated that 10 million Km^2 of the sub-Saharan African arable land covering 36 countries is infested with tsetse flies (Kuzoe, 1993 and Latif, *et al.*, 2002). The northern limit of the fly-belt is the Sahara desert and the southern limit is the Kalahari desert, Natal and the tropical highlands (Figure 1.6). The main factor that determines the distribution of tsetse flies is the vegetation (Evasion and Kathuria, 1982). Optimal temperature for tsetse fly survival is 25°C. Sub-zero temperatures and about 46°C do not favour tsetse survival (Mulligan, 1970)

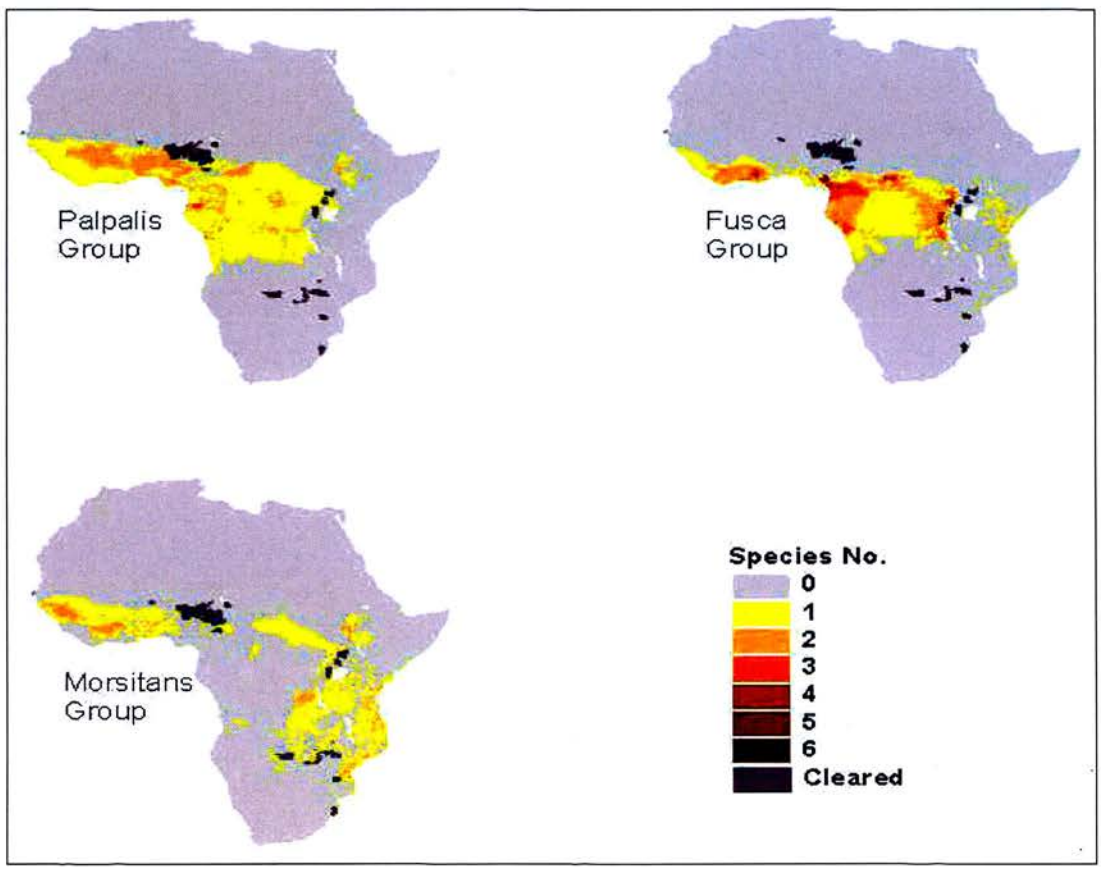


Figure 1.6 Distribution of tsetse in Africa. Soure: http://www.fao.org – FAO Atlas 1996-2000

1.4.4.2. Species of tsetse flies in Africa

The tsetse flies belong to the order Diptera, family Glossinidae and genus *Glossina* (Mulligan, 1970). Tsetse flies can be distinguished from other biting flies by their forward-pointing mouthparts (proboscis) and characteristic wing venation (hatchet cell). Based on their morphology (e.g. genital structure, size,) and habitat requirement, the genus is further divided into three groups (subgenera): The *morsitans* group (savannah group), *fusca* group (forest group) and *palpalis* group (riverine group). These groups are further divided into species and subspecies. There are 31 known species and subspecies that have been identified so far in this genus (Figures 1.6 and 1.7). Fifteen species are in *fusca* group (subgenus *Austenina*), 9 in *palpalis* group (subgenus *Nemorhina*) and 7 in *morsitans* group (subgenus *Glossina* s.s.), (Rogers and Robinson, 2004).

Genus	*Glossina*		
Group	***fusca* group** **(Female genitalia have inferior signum)**	***morsitans* group** **(Male genitalia have superior claspers)**	***palpalis* group** **(Male genitalia have inferior claspers)**
Species/ subspecies	*G. brevipalpis**	*G. austeni*	*G. calliginea*
	G. frezili	*G. longipalpis*	*G. fuscipes fuscipes**
	G. fusca fusca	*G. morsitans centralis**	*G. fuscipes martini*
	G. fuscipleuris	*G. morsitans morsitans**	*G. fuscipes quanzensis*
	G. haningtoni	*G. morsitans submorsitans*	*G. pallicera*
	G. longipennis	*G. pallidipes**	*G. pallicera pallicera*
	G. medicorum	*G. swynnertoni*	*G. palpalis gambiensis*
	G. nigrofusca hopkinsi		*G. tachinoides*
	G. nigrofusca nigrofusca		*G. palpalis palpalis*
	G. severini		
	G. tabaniformis		
	G. vanhoofi		
	G. fusca congolensis		
	G. nashi		
	G. schwetzi		

Figure 1.7 Tsetse species and subspecies (adopted from Leak, 1998). * = tsetse flies found in Zambia.

1.4.4.3. Species of tsetse flies in Zambia

Connor R.J. (1994) reported that more than five-eighths of Zambia is tsetse infested, and the boundaries of tsetse infestation are not constantly monitored, indicating boundaries are only estimates. Zambia has nine provinces and all of them are infested with tsetse flies (Figure 1.8). There are four species of *Glossina* in Zambia, namely:

Glossina morsitans (Glossina morsitans morsitans and Glossina morsitans centralis)

Glossina pallidipes

Glossina fuscipes

Glossina brevipalpis

Glossina morsitans is the most widespread species in Zambia covering approximately 213,888km^2 of the 742,400km^2 country's area (Evison and Kathuria, 1982).

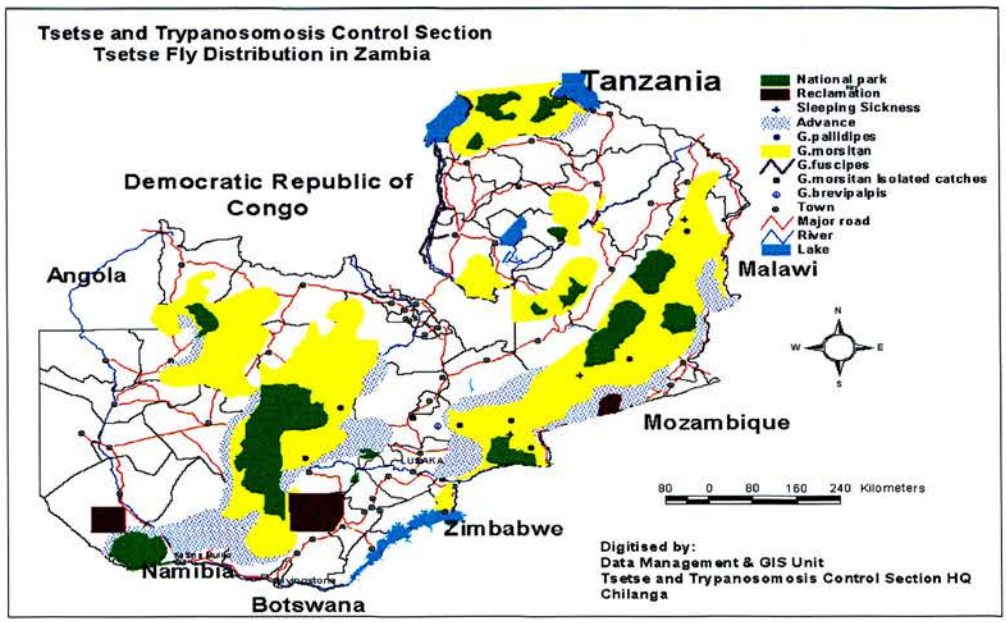


Figure 1.8 Map of Zambia showing tsetse distribution

1.4.4.4. The life cycle of tsetse flies

The female tsetse fly does not lay eggs but produces larvae, one at a time. Once the egg is fertilized, takes about 4 days to hatch. Inside the fly in the uterus, the 1st instar larva develops into 2nd instar larva and finally into 3rd instar larva that is deposited into the ground and burrows into cool moist soil. The development of 1st instar larva to 2nd instar larva takes about 1 day. The development of 2nd instar larva to 3rd instar larva takes about 2 day. In the soil, the 3rd instar larva will develop into pupa in a time period of just over 2 days (FAO, training manuel, 1982). While in the soil the pupae will develop into an adult and emerge from the ground. The time the pupa takes to develop into an adult is between 30-40 days. Female mate only once in their life and with optimal availability of food and breeding habitat, can produce a larva every 10 days (FAO, training manual, 1982; Leak, 1998)

1.4.5. Epidemiology of African trypanosomiasis

1.4.5.1. Distribution problem

Understanding the manner in which African trypanosomiasis is spread, maintained and its impact on the animal population is very important if control and prevention strategies are to be effectively implemented. Distribution of this disease is related to areas that are inhabited by tsetse flies in the tropics and sub-tropical countries of the sub-Saharan Africa. However there are cases of trypanosomiasis that have been documented where trypanosomes may be transmitted by vectors other than tsetse flies (Wells, 1972, Roeder, *et al*, 1984).

The epidemiology of trypanosomiasis is determined by several factors such as altitude (Evasion and Kathuria, 1982), climate, tsetse density, infection rate, host preference of the tsetse flies and livestock management (Connor, 1993). Connor (1993) observed that these factors that play significant roles in the epidemiology of African animal trypanosomiasis are well recognised but have not yet been fully understood.

1.4.5.2. Climate and vegetation

The overriding factor in the epidemiology of tsetse-transmitted trypansomiases is climate (Evasion and Kathuria, 1982). The seasonal changes in rainfall and temperature have been known to affect the distribution of the tsetse flies and trypanosome infection rate in animal hosts. When temperatures are high, tsetse flies will try to find cool micro-climate provided by a good vegetation cover, so prefer living in habitats that provide good shade for their resting and pupae development (FAO, 1982). Just like many other invertebrates their development is temperature and humidity dependent. In dry hot season flies concentrate in areas where there is good vegetation cover while in the wet season the flies are widely distributed (Van den Bossche, *et al*, 2002).

Although both climate and vegetation have a great effect on tsetse distribution or ecology (Nash, 1937; Ford, 1963), the only true indicator is vegetation (Evison and Kathuria, 1982). Abundance of tsetse varies according to spatial changes in the density of hosts and resting sites (vegetation). The abundance of tsetse declines from mature woodland with an abundance of hosts and resting sites, to open areas where such resources are scarce or absent (Torr *et al*, 2004). Changes of tsetse habitat (vegetation) by encroachment of people and their livestocks has significant effect on tsetse distribution and density (Van den Bossche, 2001). The concept of tsetse habit or vegetation distruction had been used to control tsetse (Mulligan, 1970).

1.4.5.3. Tsetse

Different tsetse species are found in different habitats; the *Morsitans* group in areas of savannah (grassy woodland); the *palpalis* group in the humid areas such as swamps, the rain forest, the lake shores and the gallery forests along rivers; the *fusca* group in thickly forested areas. These different habitat preferences result in different tsetse species vectoring trypanosomes in different environments. Vector capacity of flies seems to play a significant role in transmitting different parasites. Uilenberg (1998) in his studies demonstrated that teneral tsetse flies of the savannah group had higher mature infection rates of *T. congolense* (savannah type). In areas where the

tsetse population does not live long enough because of the adverse conditions that results in shortening their lifespan and death, the proportion of flies with mature *T. vivax* infections increases and that of *T. brucei* decreases. This is because the life cycle of *T. vivax* is shorter and the trypanosomes are able to mature before the fly dies. Places such as at the edge of tsetse fly-belt where the conditions are not favourable for the fly will present this situation (Connor, 1993, Van den Bossche, 1999). Different species of tsetse also show different host preference and based on this Weitz (1963) categorized them as follows (Table 1.7):

Table 1.7 Host preference of tsetse flies

	Tsetse species	**Host preference**
1	*G. swynnertoni, G austeni, G. tabaniformis, G. fuscipeuris*	Suidae
2	*G. morsitans morsitans, G. morsitans submorsitans*	Suidae and Bovidae
3	*G. pallidipes, G. longipalpis, G. fusca*	Mainly Bovidae
4	*G.longi pennies, G. brevipalpis*	Mainly mammals other than pigs and Bovidae
5	*G. palpalis, G. fuscipes fuscipes, G. tachinoides*	Most available hosts and man

Clausen (1998) observed that *Glossina austeni* and *G. fuscipleuris* fed more on Suidae (mainly bushpig) while *Glossina morsitans* species fed mainly on Suidae (mainly warthog). He observed also local variations, in some areas hippopotamus or ruminants replaced the warthog as the main host. *G. longipalpis* and *G. fusca* prefered feeding on bushbuck and *Glossina pallidipes* fed mainly on ruminants (buffalo, bushbuck and cattle). The main source of blood-meal for *G. brevipalpis* was Hippopotamus.

1.4.5.4. Animal host

Various host-associated factors such as breed and stress are known to complicate the epidemiology of trypanosomiasis in the animal host and some animal breeds possess inherent capacity to control and reduce or even self-cure infection such as the trypanotolerant Ndama and West African shorthorn (Murray, 1989 and Leperre, *et al*, 1994). Wild animals such as wild Bovidae and Suidae also have this ability, depending on the species of trypanosomes the animals is infected with, they will develop different levels of parasitaemia (Connor, 1993). When cattle are infected with *T. vivax* they will develop higher parasitaemia than when they are infected with *T. congolense* and infection with *T. brucei* give rise to only scanty transient parasitaemia (Murray, 1989). Dogs develop high parasitaemia when infected with *T. brucei* (Nwosu and Ikeme, 1992; Taylor and Authié, 2004).

Stress is an important factor in the epidemiology of animal trypanosomiasis (Connor, 1993). Animals under stress are susceptible to trypanosomiasis (Osaer *et al.*, 1999; Murray *et al.*, 2004). Stress factors include; late pregnancy, lactation, overworking in draught animals and hard-working bulls used for breeding and poor nutrition, other diseases (Fiennes, 1970).

1.4.5.5. Livestock management

Management practices can determine the challenge to which livestock are exposed, for example in areas where farmers move their animals from tsetse-free areas to tsetse-infected areas in search of water and good pasture, the challenge varies greatly (Hall *et al*, 1984). Farmers in most of the areas that are affected with trypanosomiasis are poor and in most cases do not provide food supplements to their animals. It is also common that in rainy season work oxen plough for several hours in a day and are released to go and graze late in the day. These poorly managed animals are prone more to trypanosomiasis (Osaer *et al.*, 1999; Murray *et al.*, 2004).

1.4.6. The pathology of African Animal Trypanosomiasis

Knowledge of the disease progress is important if one has to make a logical diagnosis, give proper treatment and tell the possibility of cure or recovery. In animal trypanosomiasis the progress starts from infective tsetse bite to death or recovery.

The course of the disease is variable depending on the factors associated with the breed and species of host and the parasites (Murray, *et al*, 1982). The course of the infection may be acute, sub-acute or chronic. In acute cases animals develop anaemia rapidly and lose weight and body condition over a period of three to four weeks (Losos and Ikede, 1972). Other signs in acute phase include; high body temperature of about 39°C to 41°C, weakness, abortion and reduced milk production in female animals, calves born to infected cows are stunted and weak and die within month of birth. Animals that are not treated may die and those that survive this phase start a slow recovery process and enter a chronic phase of trypanosomiasis. In chronic infection the disease runs a course of many months or years (Taylor and Authié, 2004). In this phase animals will show intermitted parasitaemia or no parasites in the blood and lymph nodes and spleen returns to normal size. In chronic infection cattle infected with trypanosomes can fully recover without treatment suggested that, the infecting trypanosomes exhaust their VAT (Variant antigen types) repertoire, including those that cross-react with metacyclics, thereby leading to both "self-cure" and subsequent immunity to homologous cyclically transmitted challenge (Nantulya et al., 1984). In chronic phase the predominant signs are anaemia, emaciation and infertility. Animals will continue to eat even though they are weak (Murray and Dexter, 1988). The progress of the disease that animals follow either in acute or chronic phase starts when infective tsetse bites a host. The infective metatrypanosomes are inoculated through the skin and under the skin they pass through a period of division and development, changing and multiplying beneath the skin in the subcutaneous tissues, where a hard swelling, the chancre may develop (Roberts, et al, 1969, Apted, 1970, Pentreath and Kennedy, 2004).The chancre is very difficulty to see in animals, develops 4-14 days after an infected tsetse bite and is usually about 2-3 cm and at times can reach 10cm in diameter and resulting in skin thickening (Pentreath and Kennedy, 2004). The parasite multiplication in the chancre

induces acute inflammation response involving plasma cells, monocyctes and lymphocytes (Gray and Luckins, 1980). After under-going a period of transformation to mature trypanosomes, the metatrypanosomes migrate from the chancre site to the lymphatic system and blood stream. The incubation period i.e. the time from the infective tsetse bite to appearance of the parasites in the blood stream varies from one week to three weeks (Kennedy, 2004). The pre-patent period (incubation period) depends on the virulence of the parasites, infective dose and the immune status of the host (Taylor and Authié, 2004).

Before the parasites are detected in the blood, lymph nodes especially those draining the inoculation site are enlarged (Taylor and Authié, 2004). During the same period the spleen is also enlarged. When the trypanosomes move from the chancre to the blood stream, the animals becomes parasitaemic, its temperature rises and anaemia develops. The severity of anaemia will depend on the virulence of infecting parasites, age, breed and nutrition status of the host (Agyemang, *et al*, 1992).

Pathological features also depend on where trypanosomes are distributed in the host. Parasites that are confined to blood and lymphatic system (Haematic group) like the *T. vivax* and *T. congolense* will result in pathological changes that are mainly confined to the blood and lymphatic systems (Banks, 1979). In the Humoral group where the *Trypanosoma brucei* species are, the parasites invade the connective tissue and body cavities in addition to the blood and lymphatic system and cause damage to the invaded organs (Losos and Ikede, 1972). The most affected organs are the spleen, lymph nodes, heart and the brain. Parasites in this group invade the Central Nervous System (CNS) and develop clinical symptoms of the CNS and on post-mortem, diffuse meningo-encephalitis is a common feature in cattle (Morrison, *et al*, 1983). Although *T. vivax* is under Haematic group, it is capable of invading the anterior chamber of cattle's eye where it increases secretion of tears which is followed by the eye becoming blue and opaque and if treated becomes clear and bright (FAO, training manual, 1982).

Emaciation and anaemia are the main pathological findings in animals suffering from trypanosomiasis. Emaciation (Figure 1.9) in animals is as a result of the disruption of the lipid and carbohydrate metabolism in ruminants (Wellde, *et al*, 1989b and

Katunguka-Rwakishya *et al*, 1995). Animals that become infected with trypanosomes have low cholesterol levels (Gillet and Owen, 1987; Mubanga 1996). This results in depleting fats in the animal bodies.

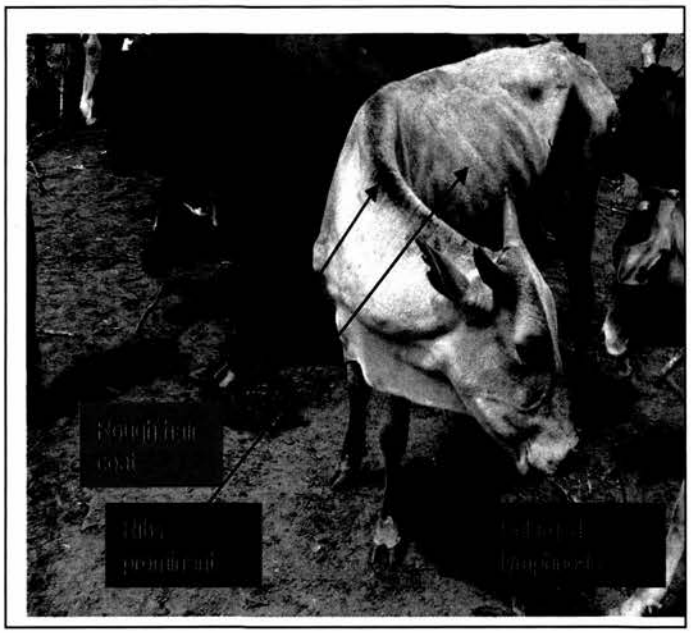


Figure 1.9 Emaciated animal suffering from trypanosomiasis

Anaemia progresses in two phases: the first phase is associated with the first wave of parasitaemia during the acute period of the disease. One of the mechanisms involved in this type of anaemia is the removal of erythrocytes from the circulation by the animal's own defence system by the process called phagocytosis (Murray and Dexter, 1988). The erythrocytes are coated with material from the lysed trypanosomes which make the red blood cells sensitive to phagocytes and mistake them for invaders (FAO, training manual, 1992). The phagocytes will engulf and destroy them. In addition to phagocytosis, the trypanosomes produce substances which destroy the erythrocytes directly during a process called haemolysis and during this process erythropoiesis is elevated but is not enough to compensate for the lost erythrocytes (Katunguka-Rwakishya *et al*, 1992).

The second phase of anaemia development takes place during the chronic phase of the disease when exotoxins are produced by the parasites as a by products that have a degenerate effect on the haemopoietic system. The red bone marrow is affected as a

result of this defect in iron metabolism affecting the production of the erythrocytes (Mamo and Holmes, 1975).

1.4.7. Diagnosis of the African Animal trypanosomiasis

The primary reason why trypanosomiasis diagnosis must be carried out is to have appropriate application of therapeutic and prophylactic or control measures (Eisler *et al*, 2004). The suitable methods preferred are those that are more economical, simple and sensitive and can be used for large-scale epidemiological studies. Trypanosomiasis is a rural problem and as such diagnostic methods must be those that can easily be used in such environment. Trypanosomiasis epidemiological studies also rely on the use of good diagnostic methods (Luckins, 1988).

Several methods have been used for diagnosing animal trypanosomiasis. These are generally put into two categories; direct and indirect methods. The direct methods depend on demonstration of the trypanosomes in the lymph or blood stream. The indirect methods identify the product of the trypanosomes or antibody produced by the host as a specific response to the presence of the parasite or its by-products.

1.4.7.1. Clinical diagnosis

Clinical diagnosis involves observing the signs that are associated with trypanosomiasis. This is achieved by clinically or physically examining the sick or suspected animals. In acute bovine trypanosomiasis the clinical signs include anaemia, enlarged peripheral lymph nodes, rough hair coat and pyrexia and in chronic phase the signs include anaemia, cachexia and infertility (Luckins, 1988, Eisler *et al.*, 2004). The severity of the disease will depend on the species of trypanosome infecting the animal, e.g. in East Africa *T. vivax* in cattle tend to cause acute haemorrhagic disease while *T. congolense* produces less acute disease (Stephen, 1986).

The disadvantage of the clinical diagnoses are that many of the clinical signs are non-specific and other diseases occurring in the same area may give the same clinical picture (Eisler *et al.*, 2004), for example malnutrition and intestinal helminthaisis

will normally show the same clinical signs as animals suffering from chronic trypanosomiasis

1.4.7.2. Parasitological diagnosis

Parasitological diagnosis involves examination of the blood or lymph fluids for the presence of the trypanosomes in the host. The method can be either **non-concentrated** or **concentrated**. Non-concentrated methods are wet blood films and Giemsa-stained thick and thin fixed blood smears. These blood films and smears are then examined for the parasites using a light microscope. The limitation of this method is that they are not sensitive enough to detect blood parasites at low parasite levels (Masake and Nantulya, 1991, Eisler *et al*, 2004).

Concentrated methods include haematocrite centrifugation technique (Woo, 1970, Woo and Rogers, 1974) and the Buffy coat technique (Murray *et al.*, 1977). The haematocrite centrifugation technique **(HCT)** involves centrifugation of blood in the microhaematocrit capillary tube and examination of the buffy coat/plasma junction under the microscope, this method is also called the Woo technique. In the Buffy coat technique **(BCT)** the capillary tube is cut and the buffy coat/plasma junction is placed on the slide and covered with a cover slip and examined for the parasites under a light microscope. In these two techniques the parasites are concentrated by centrifugation thereby making them more sensitive than the non-concentrated techniques.

The other parasitological methods include **sub-inoculation** and ***In Vitro* culture methods**. Sub-inoculation is where the blood from the suspected animals is inoculated in a naïve susceptible animal and then the animal is examined for the parasites. Xenodiagnosis is another form of sub-inoculation where the flies are fed on the suspected animals and later on dissected for the presence of the parasites. In *Vitro culture* is where culture media that has been subjected to blood from suspected animals are examined for the parasites. An example of this test is the Kit for the *in vitro* isolation of trypanosomes (KIVI) (Zweygarth and Kaminsky, 1990 and McNamara *et al.*, 1995b).

1.4.7.3. Immunological Diagnosis

These tests identify the product of the trypanosomes or antibody produced by the host as a specific response to the presence of the parasites. Under this category there are four tests namely; complement fixation test **(CFT),** Indirect fluorescent antibody test **(IFAT),** card agglutination trypanosomiasis test **(CATT)** and Enzyme-linked immunosorbent assay **(ELISA).**

IFAT involves coating the immunofluorescent slides with fixed trypanosome antigen to which the test serum is added. The fixed trypanosomes on the slide detect antibodies in the serum samples. The test measures directly the interaction between the antigen and the antibody. The reaction is observed under an ultraviolet microscope (Katende *et al.*, 1987). This test is sensitive and specific in detection of bovine anti-trypanosomal antibodies (Luckins and Mehlitz, 1978).

In the **CATT** the whole blood or serum from infected animal is mixed with antigen on a card if there is agglutination then the test is positive. This is as a result of antigen reacting with the antibodies produced by infected individual. This is more commonly for diagnosis of *T. b. gambiense* in humans although can be used for diagnosis of *T. evansi*, *T. congolense* and *T. vivax* (Luckins, 1992).

There are two types of enzyme-linked immunosorbent assays. These are the Antigen-detection ELISA (Ag-ELISA) and the antibody-detection ELISA (Ab-ELISA). In the antigen-detection ELISA the monoclonal antibody is used to coat the plate or polystyrene tubes. Test serum is added to the coated plates. The antigen in the serum is captured or trapped by the coated antibody. A second antibody, which is enzyme-labelled is added and binds to the free combined sites of the captured antigen. A substrate and chromogen are then added and the colour change is detected visually or by a spectrophotometer (Trypanosomiasis ELISA kit, Bench Protocol,Version – TRP 1.1, 1994). For the antibody detection ELISA, trypanosomes antigen is adsorbed onto polystyrene microwells that capture the circulating antibodies in the test sera. The major drawback of the Ab-ELISA is that the antibodies persist for a longer time (about 6 to 13 months) after the parasites have been cleared in the host (Luckins, 1992) and this makes it difficult to differentiate a present infection from a past infection. The Ag-ELISA, it is not very sensitive and

has been demonstrated to show cross-reactivity between *T. brucei* and *T. congolense* infections (Eisler *et al.*, 1998).

1.4.7.4. Molecular Methods for Detecting Trypanosomes

In recent years several PCR amplification techniques have been developed for detection of trypanosome species and strains. These include isoenzyme characterization-PCR (Gibson, *et al*, 1980); restriction fragment length polymorphism (RFLP)-PCR, (Hide, *et al*, 1991, Bellagamba, et al, 2001); minisatellite markers-PCR (MacLeod, *et al*, 2000); serum resistance associated (SRA) gene-PCR (Welburn, *et al.*, 2001): internal transcribed spacers (ITS) nested PCR (Cox, *et al*, 2005).

The advantage of using such PCR is that the presence of parasite DNA equivalent to one trypanosomes in 10ml of host blood can be detected and the method can also detect trypanosomes in cattle as early as 5 days after an infective tsetse bite (Maseke *et al.,* 2002). Large-scale epidemiological surveys using PCR have been made more user friendly by eliminating the use of cold chain in the field as samples can be put on the filter papers and stored at room temperature for future use (Polski *et al*, 1998, Welburn, *et al*, 2001).

1.4.8. Control of trypanosomiasis

Several methods used to control tsetse and trypanosomiasis have been developed and used in the sub-Saharan countries. Older methods included bush clearing, game fencing and elimination, residual insecticide (chlorinated hydrocarbons) application to tsetse habitat by either ground or aerial spray (Nagel, 1995). Bush clearing and residual insecticide application by either ground spray or aerial spray are not used any more because they are not environmentally friendly. In Zambia bush clearing and game elimination were used from the beginning of the century until the 1970s. Aerial spray using persistent residue insecticides was first introduced in Zambia in 1968 and remained to be used until the 1980s. Between 1968 and 1978 a total of

21,360 Km^2 was covered by aerial spray using fixed wing sequential aerial spray technique (C. Evison and K.D.S Kathuria, 1984).

The current methods are insecticide impregnated targets (Vale, 1993, Van den Bossche *et al.*, 1997), traps (Belete, *et al*, 2004), and pour-on's which have lately emerged (Hargrove, *et al*, 2000, Van den Bossche *et al.*, 2004). Zambia has been using odour-baited targets since the 1980s when Regional Tsetse and Typanosomiasis Control Programme (RTTCP) funded by the then European Economic Community (EEC) started operating in the common tsetse fly-belt covering Malawi, Mozambique, Zambia and Zimbabwe. Problems associated with vector control using targets are; vandalising, theft and very expensive to deploy and maintain. In 1998-1999, on a trial basis Pour-on was used to control trypanosomiasis in about 2000 km^2 on 20,000 cattle in Eastern province of Zambia where the monthly incidence of trypanosomal infections was reduced from a mean incidence of 12% prior to application to 0.9% (Van den Bossche *et al.*, 2004).

Sterile Insect Technique (SIT) in combination with other methods has been used to eradicate tsetse flies in some parts of Africa (Vreysen *et al,* 2000, Vale and Torr, 2005). Non-residual aerosol (synthetic pyrethroids) application by aerial spray is now used as opposed to residual insecticides in Botswana (Allsop, 1984, Kgori, *et al*, 2006). Trypanocidal drugs have also been used to treat (Machila, *et al*, 2003) and prevent the spread of disease and are still widely used in most part of the continent. The repeated use of a few trypanocidal drugs available on the market for trypanosomiasis has lead to drug resistance in most part of Africa and it is unlikely that new drugs will be made available on the market in the near future (Geerts and Holmes, 1998).

1.5. Tick-borne diseases

1.5.1. The diseases

Tick-borne diseases that include Theileriosis, Babesiosis, Anaplasmosis and Heart-water are widely distributed throughout the world, particularly in the tropics and subtropics and affect about 80% of the world's cattle population (de Castro, 1997).

Ticks transmit the parasites which cause devastating and often fatal disease (ILRI, 1991; Eisler *et al*, 2003; Aktas, *et al*, 2006). Indigenous cattle breeds often develop a certain degree of natural resistance to the parasites and in some tick-borne diseases, a passive or acquired or innate immunity is seen, under circumstances of enzootic stability (Peter, *et al,* 2005). High level of mortality has been seen primarily in exotic and crossbred cattle (Morzaria *et al*, 1988). High mortality has also been observed in indigenous calves and adult cattle in endemically unstable areas where they haven't been exposed to ticks (Perry and Young, 1995; Makala *et al*, 2003). Conserving endemic stability depends on maintaining tick populations above a certain minimum threshold (Eisler *et al*, 2003). This allows cattle to naturally become infected at an early age, when there is significant passively acquired and inmate immunity and is immune to challenge later in life (Makala *et al*, 2003). Endemic stability is said to be reached in a population when clinical disease is rare despite high levels of infections (Peter, *et al,* 2005).

1.5.2. The vector

1.5.2.1. The host

Ticks parasitize a wide range of vertebrate hosts, and transmit a wider variety of pathogenic agents than any other group of arthropods (Oliver, 1989). Ticks take their requisite blood meal from all classes of vertebrates (eg, mammals, reptiles, birds), with the exception of fish (Edlow, J.A, 2006).

1.5.2.2. Classification of ticks

Ticks belong to the phylum, Arthropoda, class, arachnids and order, Acarina, suborder Metastigmata (Peter *et al*, 2005). They are divided into two groups: soft bodied ticks (Argasidae) and hard bodied species (Ixodidae). About 899 tick species have been identified worldwide that parasitize the vertebrates (Baker and Murrell, 2004). These include: Argasidae (185 species), Ixodidae (713 species) and Nuttaliellidae (1 species). Soft ticks are resistant to starvation and can survive for many years without blood meal (Rajput et al, 2006). The economically most

important ixodid ticks of livestock in the tropical regions belong to the genera of *Hyalomma*, *Boophilus*, *Rhipicephalus* and *Amblyomma* (Jongejan, 2000). All these genera are found in Zambia (MacLeod *et al*, 1977; Macleod and Mwanaumo, 1978).

1.5.2.3. Life cycles of the ticks

Ticks' survival is dependent on a host and as such are called ecto-parasites. Ticks feed on an animal's blood or body fluids. They go through many developmental stages during their lifecycle, and feed on different species at each point. Basically they are four stages of developments which are egg, larva, nymph and adult (Minjauw and Mcleod, 2003).

Ticks begin as **eggs** (**stage 1**) that hatch into 6-legged **larvae** (**stage 2**). Larvae live and feed on animals (livestock, humans) for about a week before detaching then molting (shedding) anywhere from 1 week to 8 months later (Wall and Shearer, 1997; Randunz, 2003; McGregor, 2006). The larvae then become 8-legged **nymphs** (**stage 3**). Nymphs feed on animals, engorge for 3 to 11 days, detach, and moult about a month later (depending on the species and environmental conditions) (Wall and Shearer, 1997; Randunz, 2003; McGregor, 2006).

Once the nymph moults, it becomes an adult tick (male or female). Ticks climb up grass and plants and hold their legs up "sensing" and "looking" for their prey. Ticks are attracted to their hosts by detecting carbon dioxide and heat through special organs located on the first pair of the tick's legs (Haller's organs) (Kröber and Guerin, 1999). When a warm-blooded animal walks past, the tick crawls onto them and begins feeding. Ticks insert their mouths, attach to their prey and engorge themselves with a blood meal (**stage 4**). During feeding, tick saliva can get into the host's body and blood stream (ILRAD, 1989 Annual report). Male and female ticks usually mate while attached to the host. A few weeks later, the engorged female detaches from the host and lays her eggs (1000 – 10,000 eggs) often on the grass on the ground (Wall and Shearer, 1997; Randunz, 2003; McGregor, 2006).

1.5.3. Tick-borne diseases categorisation

Based on the vector species that transmit the parasites that cause the disease, the major tick-borne diseases are categorised into four groups (McCosker, 1979). These groups include:

1. *Boophilus* species group. In this group there is Anaplasmosis (gall sickness) which is a rickettsia disease caused by *Anaplasma marginale* and transmitted by ticks (e.g. *Boophilus spp*) and mechanically by biting flies (e.g. *Stomoxys spp*). Babesiosis (redwater) a protozoa disease caused by *Babesia* species (*Babesia bigemina, Babesia bovis*) and transmitted by *Boophilus* spp. ticks;
2. *Amblyomma* species group. Cowdriosis (heartwater) caused by *Cowdria ruminantium* a rickettsia and transmitted by *Amblyomma* spp. Apart from irritation or anaemia in case of heavy infestations, ticks can cause severe dermatitis, sometime with secondary infections of actinomycete *Dermatophilus congolensis (*Minjauw and Mcleod, 2003*)*.
3. *Rhipicephalus* spp. Group. Theileriosis (East Coat Fever, January or Corridor disease, Tropical theileriosis). East Coast Fever, January or Corridor disease is caused by *Theileria parva* and transmitted by *Rhipicephalus* spp. ticks. *Theileria annulata* causes tropical theileriosis.
4. *Hyalomma* species group. These transmit the protozoa *Theileria annulata*, which causes tropical theileriosis.

Coetzer et al (1994) classified the tick-borne diseases important to livestock industry as Theileriosis, Anaplasmosis, Babesiosis and Heartwater.

1.5.3.1. Theileriosis

The diseases caused by apicomplexan parasites of the genus *Theileria* are collectively called theileriosis. Theileriosis is a disease common in the tropical and subtropical regions of Africa, Asia and southern Europe. The parasites both in the vectors and hosts have been reported worldwide (Norval *et al.* 1992). The disease is caused by highly pathogenic species of haemoprotozoa parasites, such as *T. lestoquardi, T. annulata* and *T. parva. Theileria mutans* and *T. taurotragi* are

pathogenic but not as highly pathogenic as the first three species. The other less pathogenic species are T. *velifera*, and *T. sergenti/buffeli/orientalis* group. The non-pathogenic species include *T. ovis and T. separate* (Papadopoulos *et al.* 1996; Spitalska, *et al*, 2005). The parasites are mainly transmitted by Ixodidae ticks (*Dermacentor, Haemaphysalis, Hyalomma, Amblyomma, Rhipicephalus* species).

1.5.3.1.1. Disease caused by *Theileria parva*

Theileria parva is the causative agent of three epidemiologically different diseases, East Coast Fever (ECF), Corridor disease and January disease, caused by 3 types of *T. parva, T .p. parva, T. p. lawrencei* and *T. p. bovis*, respectively (Uilenberg, 1999). Theileriosis caused by *Theileria parva* is spread mainly by a three-host tick *Rhipicephalus appendiculatus* and is distributed in eleven countries in eastern, central, and southern African (ILRAD Report, 1991; Norval *et al.* 1992) (Figure 1.10). The other vectors include; *R. duttoni, R. zambeziensis, Hyalomma excavatum, H. dromedarii, and H. truncatum.* While buffalo and waterbuck are natural reservoirs hosts for *T. parva*, where these species do not occur the main reservoirs are traditionally-managed pastoral cattle (Perry and Young 1995; Ogden *et al*, 2003). Theileriosis has also been diagnosed in Red Hartebeest (*Alcelaphus buselaphus*) in Namibia (Spitalska, 2005). Taurine (*Bos Taurus*) cattle, their crosses, and improved Zebu (*Bos indicus*) are the most affected (Morzaria *et al*, 1988, Oura, *et al*, 2004). A carrier state of *T. parva*, defined as the persistence of a tick-transmissible infection, is common amongst naturally recovered host animals, both in cattle and major wildlife host, African buffalo (*Syncerus caffer*) (Norval *et al.* 1992).

1.5.3.1.2. Epidemiology and life Cycle of *Theileria parva*

Theileria parva the causative agent of theileriosis is transmitted mainly by three host ticks, *Rhipicephalus appendiculatus* Neumann and *Rhipiciphalus zambeziensis* Walker, Norval and Corwin throughout eastern and Southern Africa (De Vos, 1981). *R. duttoni* is also known to be the vector of the parasites in Angola. The transmission

of the disease is trans-stadial, with larvae and nymphs picking up the infection and transmitting it in their next stage of development as nymphs and adults, respectively (Mulumba *et al*, 2001). The protozoal sporozoites are produced in the salivary glands of the nymph or adult ticks and subsequently inoculated into a susceptible animal during feeding. Essentially, a tick must be attached for 48-72 hours before it transmits the sporozoites but if the environmental temperatures are high, the organism can be transmitted only in a few hours. Inside the host, the sporozoites target T and B lymphocytes (Geysen, *et al*, 1999). Schizonts may be found in regional lymph nodes within 5-8 days after inoculation. The schizont (macroschizont or Koch's blue body), causes blast-transformation of the infected lymphocyte (Figure 1.10) which produces two daughter cells, each of which contains a schizont. Every 3 days, the number of schizonts increases ten-fold. Approximately 10-25 days post-infection (average 14 days), schizonts develop into merozoites (microschizonts). These merozoites are initially found in the cytoplasm of lymphocytes, reticular cells, and macrophages. They subsequently invade erythrocytes where they become piroplasms. The piroplasm-containing erythrocytes (Figure 1.10) are ingested by larval or nymphal ticks during feeding. Once inside the gut of the tick, the protozoa undergo a sexual cycle which results in the formation of a motile stage of the organism which allows it to reach the salivary glands of the tick and the entire life cycle begins again (Figure 1.11) (Young et al, 1988). Cattle that recover from Theileria infections usually become carriers (Kariuki, 1991; Oura, *et al*, 2004).

In the Southern and Eastern provinces of Zambia bovine theileriosis is complex, with the nymphal stage of *R. appendiculatus* playing a more significant role in the transmission of the disease than was previously thought (Mulumba *et al*., 2000).

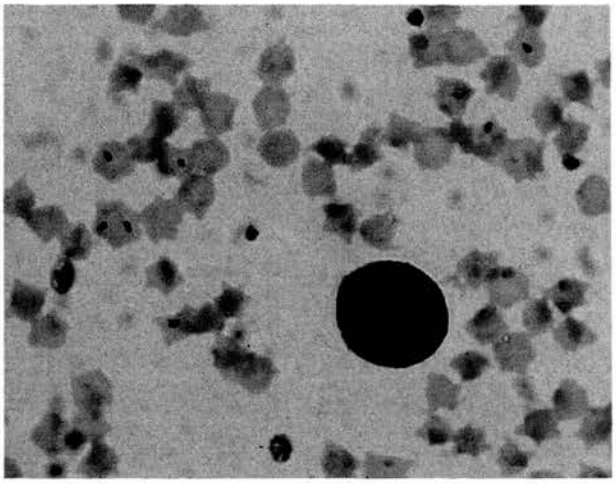


Figure 1.10 East Coast fever – *Theileria parva* . Source: University of Edinburgh® - Dr. Allan R. Walker

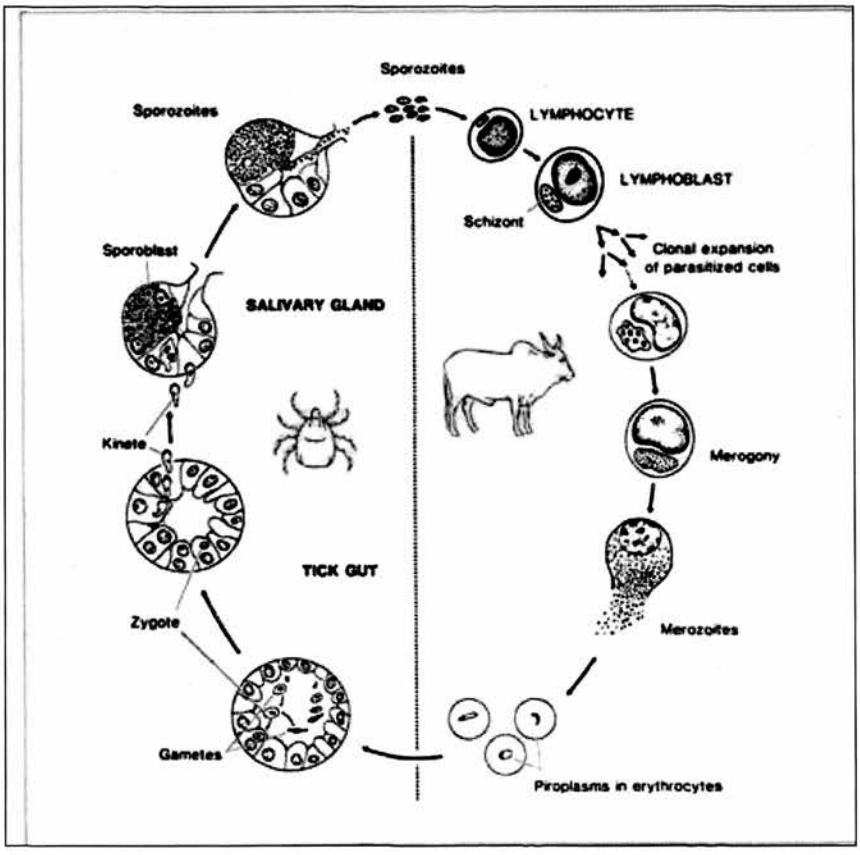


Figure 1.11 Life cycle of Theileria parasites. Source: ILRAD Annual report

1.5.3.1.3. Economic impact of *Theileria parva*.

The total regional loss due to theileriosis in 1989 was estimated to be $168 million, which included an estimated mortality of 1.1 million cattle (Mukhebi *et al.* 1992). An individual vaccination (immunisation) price ranges from US$3.30 to US$12.60 but if challenge is maintained and if vaccination is not repeated, the annual costs are low and varies in the range of US$0.25 – US$1.57 (Minjauw *et al.*, 2003). The annual cost of treating one adult animal (cattle) also varies from one country to the other and depends on frequency of treatments and ranges from US$1 - US$40 (Minjauw *et al.*, 2003). The annual cost of dipping with acaricide applied as dips, sprays or pour-on formulation is in the range of US$0.10 - US$65. The cost of dipping varies from country to country and on the frequency of application per year (Minjauw *et al.*, 2003).

1.5.3.1.4. Diagnosis of Theileriosis

Several methods have been used to detect and identify Theileriosis. These methods include; Clinical examination (Norval *et al*, 1992), microscopy (Walker *et al.* 1979; Stagg *et al*, 1981), serology (Gray *et al.*, 1980; Leemans et al. 1997, Musoke, 1993; Katende *et al.*, 1998;) and molecular biology methods – DNA probes (Allsopp et al, 1993; Geysen *et al*, 1999), polymerase chain reaction (PCR), reverse line blot hybridization (RLB) (Watt *et al.* 1997; Sparagano *et al.* 1999; Gubbels *et al.* 1999; Schnittger *et al* 2004; Odongo, 2004; McOdimba, 2005). The mostly used methods in the field is clinical examination and microscopic examination of giemsa-stained blood or lymph smears.

Clinical Examination

The initial clinical signs of theileriosis are anorexia, fever (pyrexia), enlargement of parotid lymphnodes. The other signs that appear later on are generalised lymphadenophathy, lacrimation, diarrhoea, corneal opacity, dyspnoea, frothy nasal discharge due to pulmonary oedema. A reduction in the number of white blood cells (leucopaenia) and anaemia may also be present (Siegel, 2006).

Microscopy methods

This involves the identification of the parasites in Giemsa-stained blood smears or lymph-node smears. Piroplasms in the form of signet rings or comma-shaped are occasionally seen in blood smears (Figure1.11). The schizonts are seen in lymph smears. The disadvantage of microscopy methods is that they are less sensitive when the parasitaemia is low as seen in animals that are in carrier status and this makes it of little use in epidemiological studies (Minjauw and McLeod, 2003).

Serology methods

Antibodies to *T. parva* can be detected with either an enzyme-linked immunosorbent assay (ELISA) (Musoke, *et al.*, 1993) or an indirect fluorescent antibody test (IFAT) (Minjauw and McLeod, 2003). The mostly used of the two is the ELISA because it is more sensitive and specific than the IFAT.

Molecular methods

The PCR described by Iams, *et al* (1990a), and Skilton *et al*, (2002) target the p104 associated gene in the *Theileria parva.* PCR that amplify 18S rRNA gene for the genus Theileria have also been used (Gubbels *et al*, 1999; Oura, *et al*, 2004, Spitalska, *et al*, 2005). Molecular methods have advantage over the other methods in that they are more sensitive and specific.

1.5.3.2. Control of theileriosis

Effective control of theileriosis depends on the knowledge of the distribution and dynamics of the vector (Norval *et al.*, 1992). Effective control of ticks and tick-borne diseases in livestock relies on the selection of an appropriate control method or a combination of methods (Peter *et al.*, 2005). Several methods are available for the control of theileriosis. These include; the use of anti-theilerial drugs, use of resistant breeds, immunization and vector control (Young *et al.*, 1988). Taking no action at all has also been one of the methods of controlling theileriosis, normally in very susceptible animals that are in areas where the disease incidence is high and the case-fatality is high. This allows endemic stability (a state where all calves below the age

of 6 months have been in contact with *T. parva* while clinical disease is rare) to be established in a population (Yeoman 1966; Billiouw, *et al*, 1999). It is also practiced in partially susceptible animals that are in areas where the incidence is medium and case-fatality is high or in low susceptible animals that are in areas of low incidence and low case-fatality (Perry *et al,* 1989). Restriction of cattle movement is also one of the most important means of reducing the spread of theileriosis (Makala *et al*, 2003)

Treatment with anti-theilerial drugs

Antitheileria drugs that are commonly used include parvaquone (Clexon, Wellcome), buparvaquone and the anticoccidial compound halofuginone lactate (Terit, Hoechst), which is given orally. Large doses of tetracycline given early in thc course of an infection can also treat theileriosis (Young *et al*, 1988; Merck Veterinary Manual, 1991). This method of disease control is effective in the early stage of the disease. It requires that diagnosis is done in the early stage of the disease and treatment initiated at the onset of clinical signs (Dolan *et al*, 1984). The disadvantage of using this method of control is these drugs are very expensive and their efficacy depends on rapid diagnosis of the disease as the drugs have to be given in the early stage of the disease (Young *et al*, 1988). Early and specific therapy is a principal factor in reducing the morbidity and mortality associated with these diseases (Doan-Wiggins, L., 1991).

Immunization

Control of East Coast fever by vaccination is carried out using an infection and treatment method. This is achieved by giving cattle a defined dose of live parasites (sporozoites) and simultaneous treating them with a long-acting oxytetracycline to control the infection (Radley *et al*, 1975). Two different approach are being followed: a 'local' strain approach, using a broadly protective local stock of *T. parva*; and a 'cocktail' approach, using a combination of three stocks to provide broad immunity over most of the ECF region. Detail and expensive cross-immunity trials are done to determine the strain composition of vaccine stock (Geysen, *et al*, 1999). Example of the 'cocktail' vaccine is a trivalent cocktail vaccine (Muguga cocktail) that is composed of three *Theileria parva* stocks (Kiambu 5, Muguga and the

buffalo-derived Serengeti-transformed) (Minjauw and McLeod, 2003, Oura, *et al*, 2004). However, the disadvantage of infection and treatment method is the involvement of live parasites that always relies on a cold chain and immunity is strain or stock-specific (Minjauw and McLeod, 2003). In addition severe reactions to the vaccination do occur and in some areas 5-10% of animals die (Sparagano, *et al*, 1998). Experimentally immunization has also been achieved by using recombinant antigen (p67) (Musoke *et al*, 1992; Nene *et al*, 1999). However despite great investment in research in this area, recombinant vaccines have not been sufficiently successful to see their introduction into general use.

Vector control

In much of Africa, burning of grass once a year is practiced to improve the pasture and to control tick populations. However, this has been shown to be ineffective because ticks hide in the soil and later on recolonize the area after burning (Young *et al*, 1988).

The main method of tick control at present is the use of chemical acaricides (Drummond, R.O., 1983). The acaricides that are being used for controlling ticks on livestocks include arsenical, chlorinated hydrocarbons, organophosphates, carbamates, synthetic pyrethroids (Rajput, *et al*, 2006) and formamidines (Peter *et al*, 2005). Methods that are used to apply these chemicals onto the livestock include dipping, spraying and pour on. Hand application of tick grease to the sites where ticks are attached is also another method that is used and used in cases where the tick burden is low and few animals are treated (Young *et al*, 1988; Minjauw and McLeod, 2003). Organochlorine pesticides are extremely toxic to insects and ticks, but of low toxicity to mammals. DDT is less toxic in an acute oral dose that is aspirin. Organochlorine pesticides are highly persistent in the environment and accumulate in the fatty tissue of vertebrates and can disturb hormonal systems, leading to reproductive failure. That is why they are now restricted to termite control and malaria control. Most of the chlorinated hydrocarbons have been removed from the market because they are highly toxic and have a long lifespan (Rajput *et al*, 2006).

The interval at which the acaricides are applied onto the animals is dependant on the life cycle of the ticks. One-host ticks (*Boopilus spp*) stay on the animal longer than do the 2-host tick (e.g. some *Hyalomma spp* and *Rhipicephalus spp*) or the 3-host ticks (*Rhipicephalus spp*). In this regard, the interval between dipping or spraying is much longer for the one-host ticks than the other two (Minjauw and McLeod, 2003). The interval of dipping or spraying for *Rhipicephalus spp* the causative agent of theileriosis is one or two weeks (Young *et al*, 1988).

The use of sprayers to apply acaricides to animals has many advantages over dipping, spraying equipment is highly portable and small amounts of acaricides need to be mixed for a single spray. One disadvantage of dipping is that the initial coast of a dipping tank is high and dipping of animals must be managed carefully so as to maintain the right acaricides concentration. However spraying is considered to be less effective than immersion in a dipping tank in that the chemical is not applied thoroughly on all parts of the animal body (Rajput, *et al*, 2006).

Vector control using chemicals is associated with the following problems: high cost of acaricides (Minjauw and McLeod, 2003), acquired resistance to acaricides (Eisler *et al*, 2003), destabilization of the endemic stability (Makala *et al*, 2003), environmental pollution (Minjauw and McLeod, 2003; Rajput, *et al*, 2006) and residues in animal products (Keating, 1987; Makala *et al*, 2003). The development of resistance is dependant on the volume and frequency of application of the insecticide, as well as the life cycles (Young *et al*, 1988). Development of resistance is usually seen first in the 1-host tick (*Boophilus* spp) then followed by 2-host ticks and then 3-host ticks (Young *et al*, 1988).

1.5.3.2.1. *Theileria mutans*

This species is mostly found in Africa and the Caribbean Islands and is transmitted by the ticks of the genus *Amblyomma*. *Theileria mutans* is considered to be none or slightly pathogenic though some pathogenic strains have been isolated from Kenya and Tanzania. The pathogenic strains isolated in cattle are thought to be harboured by buffalo (Moll *et al*, 1986). Unlike *T. Parva*, the piroplasm stage of *T. mutans* divides in erythrocytes of cattle and anaemia is the main clinical syndrome (Young *et*

al, 1988). The other clinical signs include; malaise, pyrexia and slight swelling of lymphnodes (Brown *et al*, 1990).

1.5.3.2.2. *Theileria velifera*

Theileria velifera is transmitted by ticks of the genus *Amblyomma* and affect cattle and buffalo in Africa. It is not pathogenic to cattle (Young *et al*, 1988).

1.5.3.2.3. *Theileria taurotragi*

This species is widely distributed in Eastern, Central and Southern Africa. The parasite is transmitted mainly by *R. appendiculatus* and in drier areas by *R. pulchellus*. The host of this parasite include; cattle, sheep, eland (Grootenhuis *et al*, 1980; Stagg *et al*, 1983) *T. taurotragi* is none or slightly pathogenic to cattle though it causes bovine cerebral *theileriosis* (Turning Sickness) of cattle.

1.5.3.3. Other tick-borne diseases

The other important tick-borne diseases important to livestock industry in the sub-Saharan African are Babesisois, Anaplasmosis and Cowdriosis.

1.5.3.3.1. Babesiosis (Red Water)

Babesia species are widely distributed in Africa and are transmitted mostly by a 1-host tick of the genus *Boophilus. Boophilus microplus* is vector for *Babesia bigemina* and *B. bovis*. *B. bigemina* is also transmitted by *B. decoloratus*. Transmission is transovarian where by transmission occurs through the next generation of larval or nymphal stages. Babesia parasites divide in the erythrocyctes. Mechanical transmission is also possible. The main clinical signs are anaemia, icterus and haematuria or haemoglobinuria hence its name Red water. The other signs include fever, in dairy cows (abortions, reduced milk yield), The disease is prominent in exotic breeds and causes few problems in indigenous breeds in area of endemic stability. Age resistance does occur where young animal are more susceptible than older ones. Infection with *B. bovis* is often acute or sub-acute and leads to death if not treated (Young, 1988; Minjauw and McLeod, 2003).

Diagnosis of Babesiosis can be confirmed by microscopy where the parasites are demonstrated in Giemsa-stained blood smears (Figures 1.12 and 1.13). Molecular methods that are species specific and sensitive have been used to confirm these parasites (Gubbels *et al,* 1999; Oura *et al*, 2004)

Treatment of Babesiosis has been achieved by the use of diminazene aceturate, amicarbalide diisethionate and Imidocarb dipropinate (Young *et al*, 1988). Control is by the use of Chemicals (dips, sprays pour on), and immunisation using a live vaccine produced from the blood of splenectomised donors (Minjauw and McLeod, 2003). Immunization against Babesiosis has been successful in Australia (Bock and Vos, 2001).

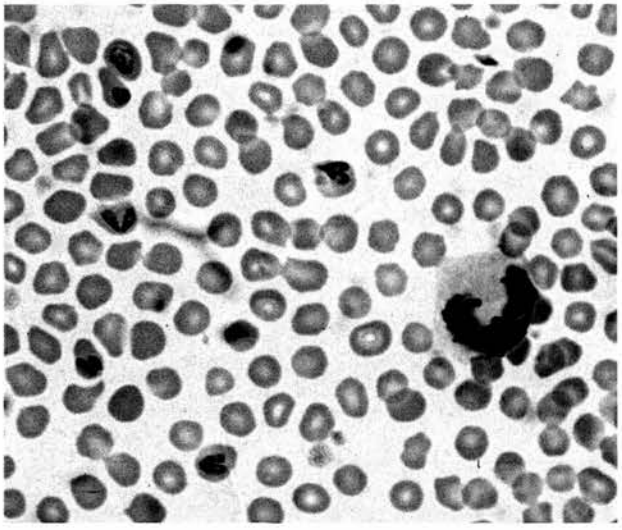


Figure 1.12 Babesiosis – *Babesia bigemina* in erythrocytes. Source: University of Edinburgh® - Dr. Allan R. Walker

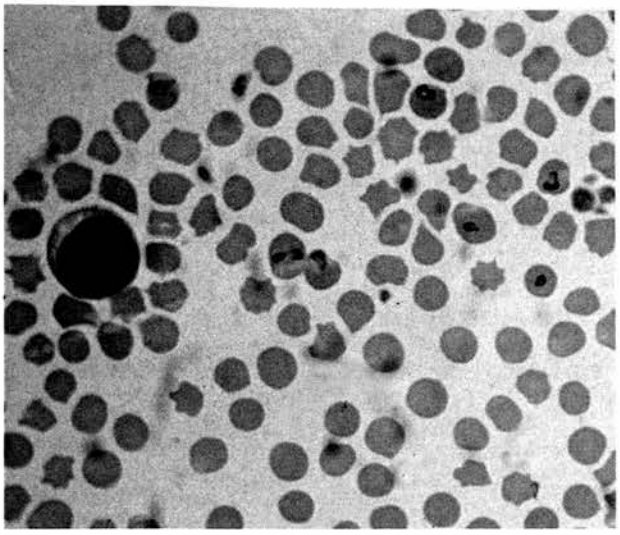


Figure 1.13 Babesiosis – *Babesia bovis* in erythrocytes. Source: University of Edinburgh® - Dr. Allan R. Walker

1.5.3.3.2. Anaplasmosis (Gall Sickness)

Anaplasmosis a rickettsial disease of cattle, sheep, goats and other wild ruminant is found worldwide and is caused by mainly *Anaplasma marginale* and *Anaplasma centrale* that are transmitted by a one-host tick of the genus *Boophilus* and mechanically by biting flies (e.g. *Stomoxys spp*). *A. marginale* is more pathogenic where as *A. centrale results in mild disease (*Ristic, 1977*)*. Anaplasma mainly affects non-resistant exotic animals though local breeds that are under poor health and condition can be affected. The clinical signs include fever, weight loss anaemia and icterus (jaundice) and up to 50% mortality (Young 1987b; Minjauw and McLeod, 2003).

The severity of the disease depends on the species involved and age of the animal. Young calves usually have an innate resistance to the disease while the acute form generally occurs in cattle from 1 to 3 years. In cattle over 3 years, the per-acute or most severe form, with rapid onset and death, predominates. Animals that survive anaplasmosis can become carriers for life and act as a reservoir for susceptible animals (Young et al, 1988).

Diagnosis is by demonstration of the causative organisms in Giemsa-stained blood smears (Figure 1.14) and the use of molecular method of a reverse line blot assay (Berker *et al*, 2002; Oura *et al*, 2004) as well as sero-diagnosis (Knowles, *et al*, 1996).

Tetracyclines (oxytetracyclines, chlortetracycline) and imidocarb dipropionate are the drugs of choice for the treatment and controlling of anaplasmosis which has to be done in the early stage of the disease. Live vaccines, Antigen-attenuated vaccines and Recombinant vaccines are used for prevention (Merck Manual, 1991; Minjauw and McLeod, 2003). Tick control by the use of chemicals has been used to control anaplasmosis.

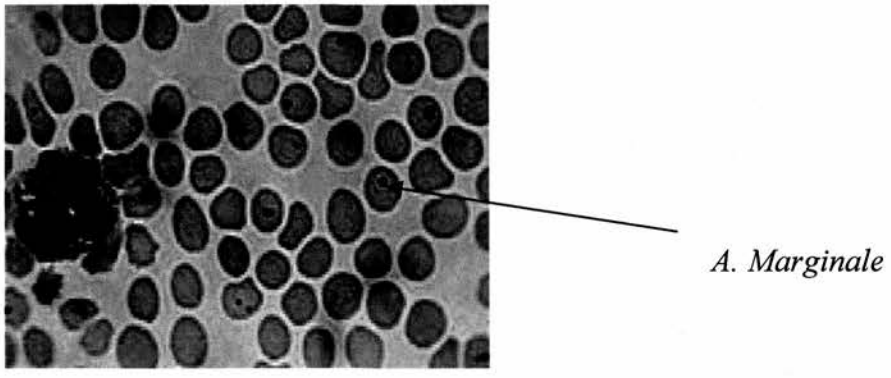


Figure 1.14 Anaplasmosis. *Anaplasma marginale.* Source: Foreign Animal Disease "The Gray Book"

1.5.3.3.3. Cowdriosis (Heart-water)

Heartwater is a rickettsial disease of cattle, sheep, goats and wild ruminants found in sub-Saharan Africa and some Carribbean island. It is caused by *Cowdria ruminantium* that is transmitted by ticks of the genus *Amblyomma (*Bell-Sakyi *et al*, 2000*).* The mechanism of transmission by *Amblyomma* species is unknown since the actual infective stage in the blood has not been determined (Young *et al*, 1988). The organism proliferates in the epithelial cells of the blood vessels of the brain and the resultant damage causes the characteristic nervous symptoms. Heartwater is most

severe in small ruminants and exotic cattle (Minjauw and McLeod, 2003). In Zambia Heartwater is mainly a disease of cattle, although outbreaks in sheep and goats have been recorded and is mainly seen in areas where regularly dipped animals are in close proximity to indigenously kept cattle with no acricidal treatment and also where game is frequently seen in cattle grazing areas (Makala *et al*, 2003)

Diagnosis is based only on demonstrating the organisms in the Giemsa-stained smears of the crushed cerebral cortex where colonies of C*owdria ruminantium* in the endothelial cells of the capillaries are seen (Figure 1.15). The other methods of diagnosis include IFAT and ELISA but these are known to cross-react between *Cowdri*a antigent and antibodies to *Ehrlichia* species (Minjauw and McLeod, 2003) and PCR methods (Bell-Sakyi, *et al*, 2000).

Control of the disease depends on tick control by intensive acaricide application, oxytetracycline treatment in the early stage of the disease and use of a live vaccine with virulent sheep's blood infected with Ball 3 stock of C*owdria* (Minjauw and McLeod, 2003).

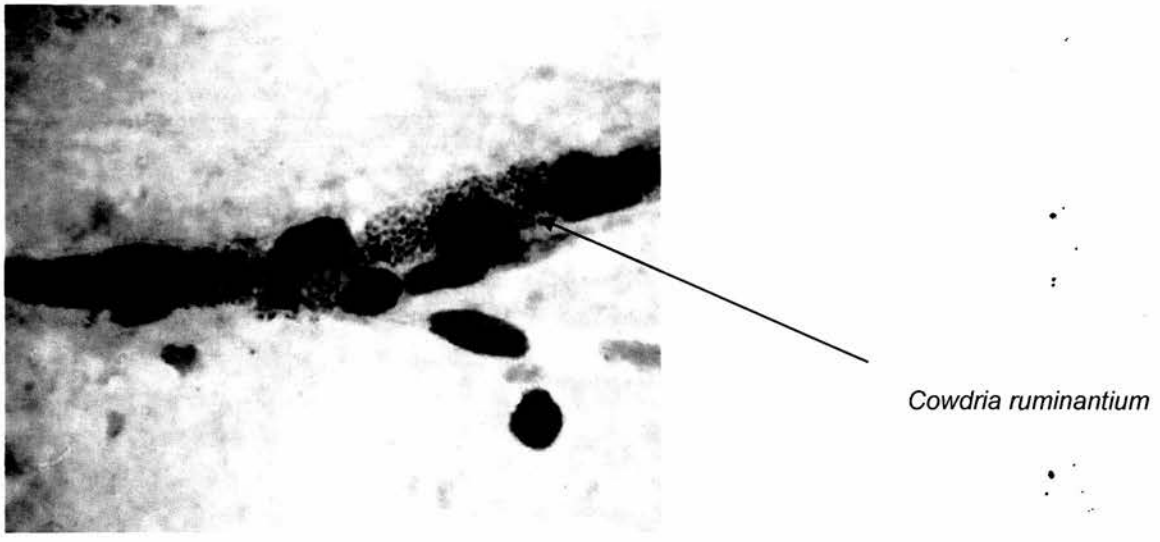


Figure 1.15 Heart-water – Brain smear from a goat. Colonies of *Cowdria ruminantium* are granular blue areas in the cytoplasm of the capillary endothelial cell. Source: Foreign Animal Diseases "The Gray Book"

1.5.3.4. Tick-borne diseases in Zambia

1.5.3.4.1. History of Theileriosis in Zambia

There are four major tick-borne diseases in Zambia. These are theileriosis (ECF and Corridor disease), babesiosis, anaplasmosis and heartwater. Theileriosis in local language is commonly known as Denkete in Southern province and Chigodola in Eastern province. Theileriosis is the most important tick-borne disease (Table 1.8)

Table 1.8 Major tick-borne diseases in Zambia for the period 1994-2004

	Theileriosis		Babesiosis		Anaplasmosis		Heartwater	
Year	**Cases**	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**
1994	11832	5402	256	87	1,652	475	143	67
1995	11,864	5,393	235	85	1,664	464	160	63
1996	11,927	5,417	334	105	1,729	492	251	104
1997	7,457	4,516	15,560	253	2,669	753	646	312
1998	1,1957	5,430	21,291	155	1,779	510	251	104
1999	9,520	4,526	10,454	253	1,782	531	380	142
2000	3,678	1,443	802	70	690	404	231	104
2001	5,885	2,279	102	20	976	233	167	35
2002	8,294	4,439	236	53	1,832	1,130	270	79
2003	6,574	2,439	*	*	*	*	*	*
2004	2,336	782	299	168	1,083	191	188	21
Total	**166,024**	**42,066**	**49,569**	**1,249**	**15,856**	**5,183**	**2,687**	**1,031**

Source: DVDL, Annual Reports. * = figures not available.

In Zambia theileriosis is restricted to Northern, Southern and Eastern provinces. In the country the first recorded case of theileriosis (East Coast Fever) was in Nakonde (formerly known as Fife) District in Northern Province in 1922 (Turnbull, 1926; Nambota *et al.*, 1994). It is thought to have been introduced into the country from East Africa where it was endemic (Thomas 1999). After the cattle population was decimated by rinderpest in 1896-1898 and the Anglo-boer war of 1899-1902 there

was need to restock cattle in the Transvaal (South Africa) and Southern Rhodesia (Thomas 1999). Cattle were shipped from Tanzania through Mozambique and Malawi and it was during this period that the disease is thought to have been introduced into Central and Southern Africa. In Eastern province of Zambia it was first recorded in 1947 in Chipata District (Nambota *et al.*, 1994). From that time the disease has been spreading south and west wards in the two provinces and has now become endemic in most part of these provinces. Theileriosis first appeared in Southern province in 1977-1978 (Anon., 1986, Minjauw *et al*, 1998) and has since spread northwards to Lusaka, Central and Copperbelt Provinces (Makala *et al*, 2003).

A survey that was conducted in the wet season in Eastern Province in 1976 revealed the incidence of blood forms of *Theileria* to be 10% in Chadiza (eastern most district), 6% in Katete and 1.5% in Petauke (western-most district) while that of Kochs's Blue Bodies was 2.3%, 1% and 0% in the same order (Veterinary and Tsetse Control Annual Report. 1976). In 1976, in Petauke District, 18 cattle were reported dead in Chief Nyanje's area between the period July to November. This occurred after the rainy season when no deaths were normally experienced.

The spread of the disease had been due mainly to poor dip management, irregular dipping and non co-operation from cattle owners. Illegal cattle movement had also lead to the spread of the disease into new areas (Veterinary and Tsetse Control Annual Reports. 1974 and 1975). In the Eastern province the spread was also compounded by the following reasons:

(a) In the Eastern province cattle have to pass through maize fields on their way to the dip tanks in the rainy season. The most important time to dip cattle is in the rainy season and this coincided with the growing of maize.

(b) Young children herding cattle in most cases purposely did not reach the dipping tanks because they spent most of the time playing on the way.

(c) The efficiency of dipping was severely curtailed when cattle covered with mud plugged into the dip tanks.

(d) Presence of streams full of water prevented cattle from going to the diptanks (Veterinary and Tsetse Control Annual Reports. 1980).

(e) While deaths from ECF in cattle may be an incentive to dip them, cessation of death in cattle resulted in cessation of dipping.

1.5.3.4.2. Control Methods of Theileriosis in Zambia

From the early part of the last century to 1983 control of theileriosis has mainly been by intensive dipping, spraying (using arsenical compounds) and stock movement restriction. In the early part of the last century it also involved isolation and slaughter of the group of cattle in which the disease had occurred (Veterinary Department Annual Report. 1941). For example, in 1941 when the disease (ECF) outbreak was reported in Isoka and Chinsali District of Northern province, a group of cattle in which it occurred was isolated and slaughtered (Veterinary Department Annual Report. 1941). From 1983 to date immunization of calves by the infection and treatment method has been extensively used in the Eastern Province of Zambia to control ECF. Since November 1987, cattle in Eastern province have been immunized using a local stock (*T. parva* Katete) on a large scale in the area (Marcotty *et al.*, 2002). In 1985 the Food and Agriculture Organisation (FAO) of the United Nations, started a theileriosis immunization programme trial in the Southern province using the Muguga cocktail and a local isolate, *T. parva* Mandali (Uilenberg, 1999). Currently a local strain *T. parva* Chitongo is being used in Southern province. Belgium Animal Disease Control Project (BADCP) that later changed its name to Assistance to Veterinary Services of Zambia Project (ASVEZA) carried out the vaccinations in Eastern province and later on took over the vaccinations in Southern province from FAO. The number of calves vaccinated and cattle dipped are shown in the Table 1.9.

Table 1.9 Number of cattle vaccinated and dipped in the period 2000-2004 in the whole of Zambia

Year	Number of Calves vaccinated	Number of cattle dipped
2000	9,729	80,066
2001	21,006	382,386
2002	21,432	294,366
2003	876	80,066
2004	676	171,615
Total	53,719	1,008,499

Source: Department of Veterinary and Tsetse Control Annual Reports 2000-2004.

Despite these methods working, they have limitations. The use of acaricides to kill ticks is hampered by long distance to dip-tanks, acaricide resistance in ticks and rising costs of the chemicals, as government that used to provide the chemical free of charge no longer controls the dip-tanks. Since 1991, the financial responsibility for dipping cattle for tick control was shifted from the government to private livestock owners (Young and Haantuba, 1997). The infection and treatment method has its own limitations that include: immunized animals remain carriers and can constitute a risk for spreading the disease into ECF-free areas where the vector is present, immunization of cattle during the incubation of naturally contracted ECF does not prevent the disease (Uilenberg 1999) and live vaccines are dependent on cold chain facilities that constitute an obstacle in rural places of Zambia where the vaccine is most needed (Makala *et al.*, 2003).

1.6. National Parks and Game Management Areas (GMAs)

It would be inconclusive of the discussion of the tsetse- and tick-borne diseases if National Parks (NP) and Game Management Areas were not discussed. These areas harbour wild animals that act as a reservoir of most the parasites of tsetse- and tick-borne diseases. In Zambia, tsetse flies are mostly restricted to National parks and GMAs and areas adjacent to them (Figures 1.1., 1.6 and 1.16)

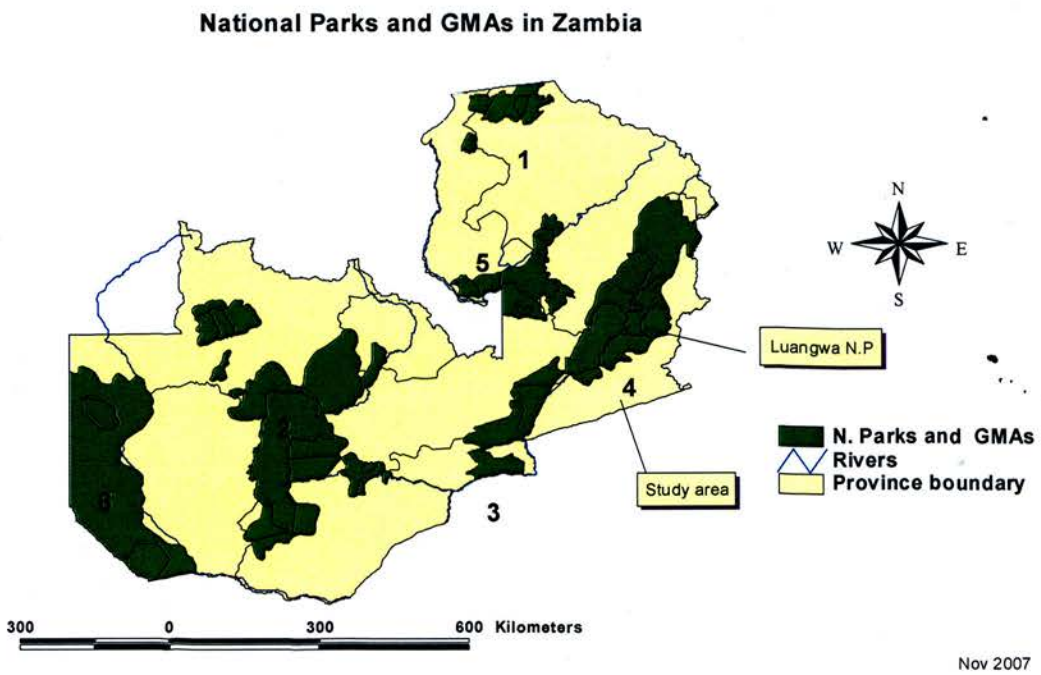


Figure 1.16 National Parks in Zambia and their surrounding Game Management areas. The study area and the Luangwa National Park are highlighted. Number 1-6 represent the game management systems.

There are 19 National Parks and 35 GMAs in Zambia. In the National Park no human settlements and game hunting is allowed while in the GMAs, human settlements are allowed and game hunting is only authorized by Zambia Wildlife Authority (ZAWA). The GMAs and NP are organised into six wildlife systems in Zambia. These are; (1.) Mweru Wantipa in the North (Luapula) with 2 GMAs and 3

NPs, (2) The Kafue system in the central part of Zambia with 15 GMAs and 4 NPs, (3) The Lower Zambezi system in the southern part with 3 GMAs and 1 NP, (4) Luangwa system in the eastern with 7 GMAs and 5 NP, (5) The Bangweulu system in the northern part of Zambia with 6 GMAs and 3 NP and (6) The Western Zambezi system in the western with 2 GMAs and 3 NPs. The Luangwa has more NP than the other wildlife systems in the country (ZAWA database office, Chilanga).

In Zambia distribution of tsetse flies are largely restricted to NPs, GMAs and areas that are adjacent to them, therefore domestic animal trypanosomiasis is a problem in these areas. It is because of this relationship that consideration of NP and GMAs is an important component of tsetse and trypanosomiasis control. As a result of this the Department of Tsetse Control and the Department of Game operated as one entity i.e. was one department from 1942 to 1959. It was called the Department of Game and Tsetse Control. Working as one department made it easier for tsetse control operations. For example when the methods of game elimination and ground spray using insecticides were used to control tsetse flies in Zambia the permission to go ahead with the operations was much faster. Today this situation no longer exists and obtaining the permission to carry out operation or research work in NP can hamper operations. The Department of Veterinary and Tsetse Control now has to go through Zambia Wildlife Authority (ZAWA) to issue a go ahead permit for tsetse operation or research work to be done in these parks.

The South Luangwa National Park and the GMAs in Eastern province are among the best administered management areas in the country and this has lead to the increase in the wildlife population in the Luangwa valley resulting in more tsetse flies. In the Luangwa valley poaching has been controlled by the introduction of village scouts (ZAWA, 1998). These are people who are recruited among villagers and live in the villages. The presence of village scouts makes it difficult for the people to poach wild animals. These village scouts report any suspicious activities directly to Wildlife Authority personnel. The Wildlife personnel pursue any dubious activities based on the information given to them by the village scouts. These NPs and GMAs in Eastern province have been well managed because of the numerous projects that

the Department of Game (ZAWA) and donor communities have undertaken in the area.

The rinderpest pandemic of 1896-1898 (Thomas, 1999) that decimated the population of livestock and susceptible wildlife in Africa did not spare game animals in this area. During this pandemic a lot of wild animals died and this resulted in reduction of both the size of the tsetse belt (Appendix 2) and tsetse density, as most of the food source were removed from the parks (Thomas, 1999). The Zambia Wildlife Authority's control measures put in place to control poaching have lead to an increase in some wildlife species. This has had a direct effect on the tsetse population in the adjacent farming areas as wildlife animals carry tsetse flies from the National Parks and GMAs to the human settled area as they look for food and water. Game fences previously established along the southern boundaries of the GMAs to control animal movements in the Luangwa Valley are no longer there. Wildlife and the tsetse populations that feed on them are free to migrate from the NPS and GMAs to the human settled areas as they look for food and water. Since the first human case was documented in 1909 (Buyst, 1977) cases of both domestic livestock and human trypanosomiasis have been diagnosed from the Luangwa valley (Figure 1.5 and Tables 1.4 and 1.5).

1.7. Thesis objective

1.7.1. Overall objective

The overall objective of this study was to look at the epidemiology of trypanosomiasis from the Luangwa valley to the plateau in Eastern Province, Zambia with the aim of identifying factors that are responsible for the spread and maintenance of the disease in a population in this area. The effect of the restricted application of insecticide deltamethrin spray (decatix®) on cattle to control tsetse and tick-borne diseases (theileriosis) was also evaluated.

It is hypothesised that factors such as combination of livestock species and type kept, altitude, cotton growing and family sizes influence the epidemiology of domestic animal trypanosomiasis in the Eastern Province, Zambia. It is also hypothesised that

applying insecticides to preferred tsetse flies feeding sites on the cattle can control the trypanosomiais and at the same time control tick-borne diseases.

To test these hypotheses the activities listed under specific objectives (section **1.7.2** below) were conducted in the study area.

1.7.2. Specific objectives

- To determine the relationship between disease prevalence and household size, altitude and livestock/crop farming practice.

- To determine the species of trypanosomes found in the livestock in the area and distribution patterns from the valley to the plateau.

- To evaluate the effect of the restricted application of insecticide deltamethrin spray (decatix®) on cattle to control tsetse and tick-borne diseases.

1.8. Study design

The study design integrated several different components. The major components were structured as a series of tasks that needed to be accomplished to achieve the overall objectives, as follows:

- Collecting historical data on both human and animal trypanosomiasis in the area.
- Collecting historical data on Game Management Area (GMAs) in Luangwa Valley, Zambia.
- Conducting a human and agriculture census in the study area in Mambwe District in the Luangwa valley, Eastern province.
- Carrying out a human and agriculture survey by using a standardised questionnaire.
- Conducting a cross-sectional study survey from the valley to the plateau.

- Conducting a longitudinal study to evaluate the effect of the restricted application of insecticide deltamethrin (decatix®) on cattle to control tsetse-borne and tick-borne diseases in Petauke District.

For greater details of the study design, see Chapter two and the respective chapters covering each activity mentioned above.

1.9. Relevance of the study

The outcome of the study will help in determining the disease pattern and that will in turn help in understanding the epidemiology of trypanosomiasis in this area from the valley to the plateau. The plateau is well settled and cultivated while in the valley the human and livestock populations have just started increasing as a result of people immigrating into the area in search of fertile land for farming. Furthermore the result of the work on the restricted application of insecticides on cattle to control tsetse- and tick-borne diseases will provide tsetse control policy makers and all stakeholders with information that will help them come up with effective control strategies for human and animal trypanosomiasis as well as tick-borne diseases in the area.

1.10. Thesis layout

Chapter One: This chapter covers literature review on tsetse- and tick-borne diseases and general introduction. A review of tsetse- and tick-borne diseases in Zambia is also covered in this chapter.

Chapter Two: General Materials and Methodologies that were used in the study in the proceeding chapters are outlined in this chapter.

Chapter Three: This chapter looks at the human and agriculture census that was done in Mambwe District, Luangwa Valley in Eastern Province, Zambia, and village characterization were based on the out-come of this census.

Chapter Four: Chapter four looks at the cross-sectional animal trypanosomiasis survey that was done starting from the base of the valley to the plateau to look at the factors that influence the epidemiology of the disease in the area.

Chapter Five: Prevalence of tsetse and tick-borne diseases in Petauke District (baseline data)

Chapter Six: Chapter six covers the work that was done on the study of the effect of restricted application of insecticide on cattle to control tsetse- and tick-borne diseases.

Chapter Seven: General discussion, Conclusion and Recommendations

- References of all the literature cited in the thesis.
- Appendices

2. CHAPTER TWO

2. GENERAL MATERIALS AND METHOGOLOGIES

2.1. Specific methodologies and materials

This chapter covers general methodologies and materials that are common to the chapters in this thesis. Specific methodologies and materials are covered in their respective chapters.

2.2. Study Area

Zambia with a land area of 752,620 Km^2 is land-locked and has eight neighbouring countries The country is divided into nine provinces and these provinces are further subdivided into several districts (Figure 2.1). The country's livestock population in the year 2004 was cattle (2,341,970), goats (1,002,379), sheep (97,605) and pigs (286,726). Out of this livestock population 190,671 (cattle), 28,737 (sheep), goats (261,705), pigs (143,963) are found in Eastern province (DVLD, Annual Report, Zambia, 2004).

2.2.1. Climate

Zambia has three climatic seasons: hot and dry season from September to October, warm and wet season from November to April, and cool and dry season from May to August (Van den Bossche, *et al*, 2004). The average rainfall is 1400mm in the northern part of the country and 600mm in the southern part. The mean minimum temperatures in June/July are in the range of 5-13°C and mean maximum temperatures in October are 25-35°C. The highest temperatures are recorded in the Zambezi and Luangwa valleys (Pegram, *et al*, 1986).

2.2.2. Vegetation

Savannah woodlands cover almost 80% of Zambia; the remaining area is covered by grassland and swamps. The savannah woodlands include; Miombo (*Brachystegia* and *Julbernardia* tree species) woodlands, Munga woodlands (*Acacia*, *Combretum*, *Terminalia* species), Mushibe woodlands, Mopane woodlands (*Colophospermum mopane*), Chipya woodlands and Lusese woodlands. Munga woodlands make up 60% of the woodlands (Pegram, *et al*, 1986).

The study area in the Eastern Province covered both the plateau and the valley. The plateau gently slopes down to the Luangwa Valley with no steep escarpment and there is no topographical barrier between the valley and the plateau. On the plateau original vegetation has been cleared for agriculture while in the valley the vegetation is relatively undisturbed as there are few settlements. The plateau is dominated by 'Miombo' woodland (*Brachystegia*, *Julbernardia* spp) and 'Munga' woodland (*Acacia*, *Combretum*, *Terminalia* species). 'Miombo' woodland is a two-storied fairly closed woodland with sparse glass cover and is mainly found on poor soils. 'Munga' woodland is a one- or two-storied fairly open woodland with a richer grass cover and found mainly on better soil types. The vegetation gradually changes to 'Mopane' woodland a one-storied woodland found in the lower-lying valley. Grassland dominated by grasses with little or no tree or shrubs can be seen on the plateau and in the valley.

2.2.3. Location

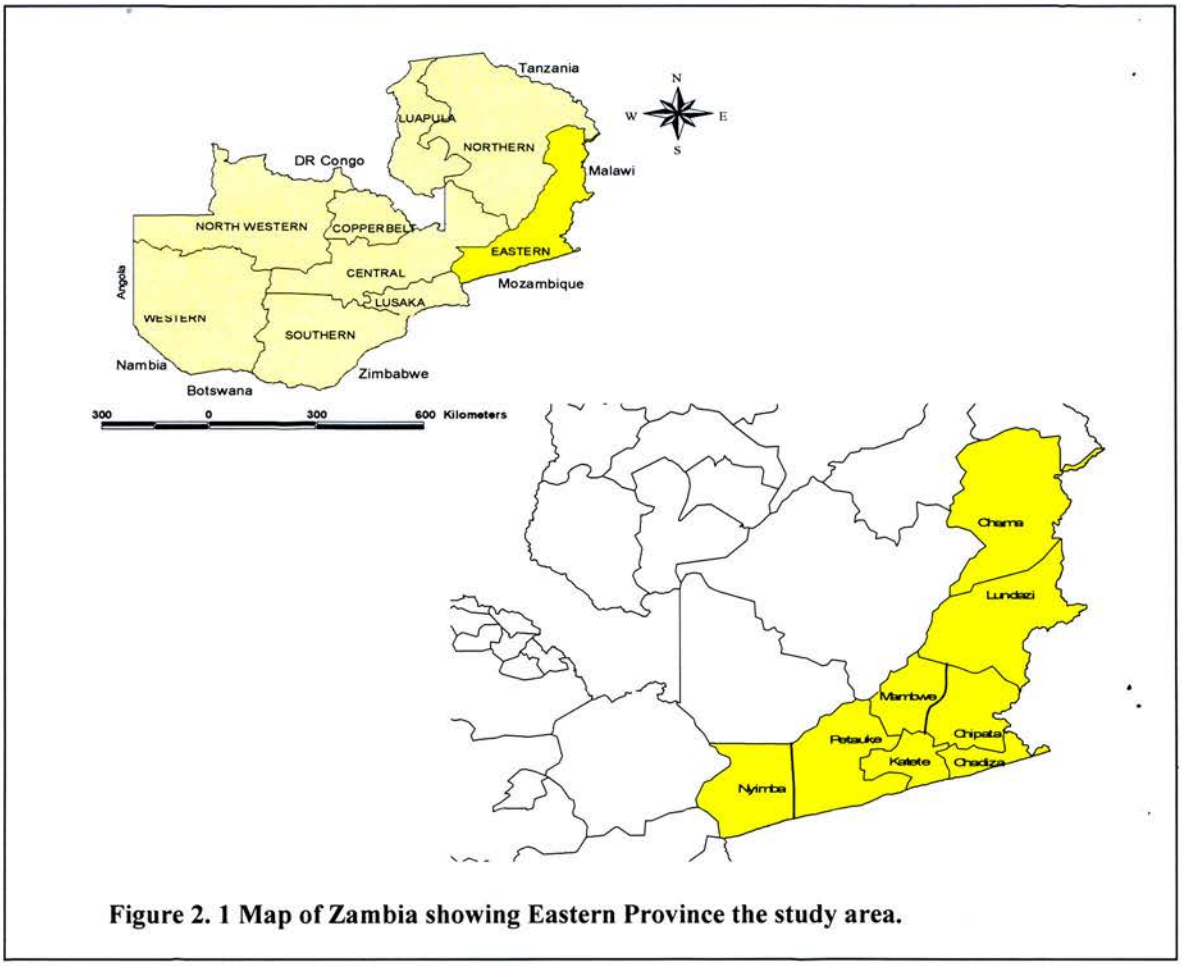


Figure 2. 1 Map of Zambia showing Eastern Province the study area.

The study area is Eastern Province, Zambia and lies between longitude 30° 48'E - 31° 48'E and latitude 13° 06'S - 14° 42'S (Figure 2.1). This province is bordered by Malawi to the east, Mozambique to the south, Lusaka Province to the west and to the north by Central Province and Northern Province. Eastern Province has eight districts: Nyimba, Petauke, Katete, Chadiza, Chipata, Mambwe, Lundazi and Chama. The study area covered three districts of the province i.e. Nyimba, Petauke and Mambwe Districts. The area is tsetse infested and many trypanosomiasis cases have been reported in animals (Mubanga, 1996; Machila *et al.*, 2001; Sinyangwe *et al*, 2004).

Much of Petauke and Nyimba Districts lie on the plateau (1000m above sea level) part of the study area. The area is highly cultivated and well settled by people. The cattle density on the plateau in the area was 5 head of cattle/km^2 as reported in 1997 (Van den Bossche and Staak, 1997) but in recent years this has increased to10 head of cattle per km^2 in 2004 (Van den Bossche *et al*, 2004).

Theileria species found on the plateau are *Theileria parva parva*, *T. mutans*, *T. velifera* and *T. taurotragi.* The other tick-borne disease parasites that have been recorded on the plateau are *Babesia bigemina, B. bovis, B. canis*, *B. cabale*, *Anaplasma marginale, A. centrale* and *Cowdria ruminantium*. The common vector of the tick-borne diseases that have been demonstrated on the plateau include *Rhipicephalus appendiculatus, R. zambeziensis*, *R. evertsi*, *R. sanguineus*, *Boophilus microplus, B. decoloratus, Amblyomma variegatum, A. hebraeum (*Makala *et al*, 2003*)*.

The main species of tsetse flies found in Eastern Province include; *Glossina morsitans morsitans*, *Glossina pallidipes* and *Glossina brevipalpis (*Evison and Kathuria, 1982). *G. m. morsitans* is the only species of tsetse found on plateau. The common species of trypanosomes circulating in the livestock in the area are *Trypanosoma congolense*, *T. vivax* and *T. brucei (*Mubanga, 1996; Machila *et al.*, 2001; Sinyangwe, *et al*, 2004).

Mambwe District is situated in the Luangwa valley. Most of the district lies in the valley and semi plateau area between 510m and 900m above sea level with peaks reaching up to 1100m. The district with a land mass of about 4,840 Km^2 is divided

into two main farming systems, the valley and semi plateau area. The semi-plateau is characterized by sand-loam soils while the valley is made up of alluvial soils that are quite fertile but prone to erosion. Most of the district lies in the Game Management Area (GMA). The valley is part of the Great Rift Valley of East Africa. The main crops grown are maize, rice, sorghum, groundnuts, cowpeas, sunflower, cotton, tobacco, cassava and potatoes. Livestock reared in the area include, cattle, goats, sheep, pigs, chickens, doves, guinea fowls and donkeys (Mambwe District, First quarterly Report, 2005). Just like on the plateau the inhabitants are subsistence farmers. Human and domestic animal population in the valley is low due to the presence of tsetse flies.

2.3. Sensitization of Stakeholders

Before the study commenced all stakeholders in the study area were informed about the sampling of the animals in the area. The stakeholders included village chiefs, village herd-men, local farmers and government staff from the Department of Agriculture and Veterinary. The information was disseminated to the stakeholders through meetings that were held at villages and government offices. The meetings before the programme started were necessary because it was important that people were fully aware of the study planned in their areas and what their roles were in the study. A schedule of the work programme was also outlined to them during these meetings.

2.4. Demographic and social surveys

Demographic characteristics and livestock herd structure in recently-settled area in Mambwe District were done to identify the households to be interviewed and the sample size of the animals to be sampled during the cross-sectional study that was conducted in the area. This was achieved by conducting animal and household census in the study area in Mambwe District from the base of the valley to the

plateau (chapter 3). Household demographic characteristics and livestock herd structure were determined by the use of a questionnaire (chapter 3).

2.5. Geo-referencing of crushpens and households

All crush-pens and households where animals were to be sampled were geo-referenced using an Etrex 12 channel Ground Positioning System (Garmin, Taiwan). All the households that were in the study area in the Luangwa valley were also geo-referenced. The GPS gave positions of these places in terms of latitudes, longitude and altitude (height) above sea level. The positions were downloaded onto a computer using the MapSource version 6.10.2 (Garmin 1999-2006, Garmin Ltd) software programme and later on maps were produced using ArcView GIS version 3.2 software programme (Environmental Systems Research Institute, Inc).

2.6. Sampling frame

The number of animals sampled are discussed in their respective chapters.

2.7. Clinical examinations

The cattle were clinically examined in the field; particular attention was paid to the lymph nodes, condition of the skin, body condition, diarrhoea and presence of lacrimation. These are some of the clinical signs that are mostly presented by animals that are suffering from tsetse- and tick-borne diseases. All the clinical signs were recorded on the field record sheets (Table 2.1). In Peatuke District where a longitudinal study was done, animals were ear tagged but in Mambwe district were not and only animal names were used for identification.

Table 2. 1 Field record sheet

SHEET...OF...

TRYPANOSOMOSIS SURVEY FIELD RECORD SHEET, YEAR 2005 DFID PROJECT, EASTERN PROVINCE, ZAMBIA

COUNTRY:..... DATE:.....
DISTRICT:..... RECORDER:.....
SAMPLING SITE:..... LATITUDE:.....
VET CAMP:..... LONGITUDE:.....
CHIEFDOM:..... ALTITUDE.....

	TAG NO	ANIMAL OWNER	BREED	AGE	SEX	WEIGHT	BODY CONDN	HB	TREATMENT	COMMENTS
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										

2.7.1. Weighing of the animals

The weight of each animal in the study was determined by using a weighing band (CEVA, SANTE ANIMALE, France) for the purpose of drug administration and monitoring of condition. In order to weigh the animal, the band was placed just behind the hump and over the chest as shown in Figure 2.2. The weights were taken either in a crush-pen or a kraal where crush-pens were not available. In places where both were in absent, the animals were cast down and measurement done while the animal were in lateral recumbency.

Figure 2. 2 Weighing in a kraal using a weigh band in Luangwa Valley, Zambia

2.7.2. Lymph node condition

The femoral, pre-scapular and the parotid superficial lymph nodes of cattle were physically palpated. They were classified according to how much they were enlarged. If they were not enlarged they were classified as normal and depending on how much they were enlarged as mild, moderate or severe.

2.7.3. Condition of the coat

The condition of the cattle coat was recorded as either normal or starring. The animals that had hair that was rough, standing and not shinny was regarded as having a starring coat. Young animals sometimes have coats that are starring even if they are healthy and because of this it was important to consider this when making a diagnosis.

2.7.4. Body condition scoring

Condition scoring of cattle was done visually; using the method originally described by Nicolson and Butterworth (1986) in a guide to condition scoring of Zebu cattle. Nine scores were used in which the three main conditions fat [F], medium [M] and

Lean [L], were subdivided into three categories. The scores were abbreviated as F+, F, F-; M+, M, M-; L+, L and L-. These scores described the condition of the animal from very thin (L-) to very fat (F+).

2.7.5. Lacrimation

Based on the observation of lacrimation this condition was classified as Yes or No; yes when there was discharge from the eyes and no when discharge was not observed.

2.7.6. Diarrhoea

This was recorded as diarrhoea observed in cases where the animal had waterly faeces.

2.7.7. Haemoglobin (Hb) reading

The haemoglobin values were measured using HemoCue Hb 201^+ analyser (HemoCue AB, Ängelholm, Sweden) (Figure 2.3). Blood (10µL) from the periphery of the ear vein was collected into a polystyrene HemoCue Hb 201 Microcuvette (HemoCue AB, Ängelholm, Sweden) cavity. The microcuvette cavity contains reagents (40 % w/w Sodium Deoxycholate, 18 % w/w Sodium azide, 20 % Sodium Nitrite, 22 % w/w Nonreactive Ingredients) deposited on its inner walls. The blood sample is drawn into the cavity by capillary action and is mixed spontaneously with these reagents. The reagents release haemoglobin from the erythrocytes and finally form azidemethaemoglobin reaction. The azidemethaemoglobin reaction is read by placing the microcuvette in a HemoCue Hb 201^+ analyser (HemoCue AB, Ängelholm, Sweden) in which the transmittance is measured and the haemoglobin level calculated. This method provides immediate and accurate results covering the whole measurement range of 0-25.6 g/dl (0-256 g/l or 0-15.9 mmol/l)

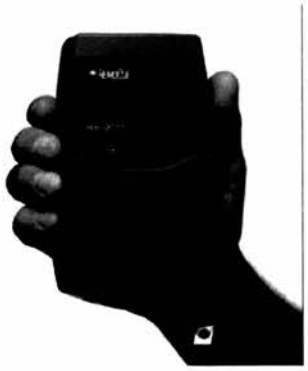


Figure 2. 3 HemoCue Hb 201^{+} analyser. Source: HemoCue® Hb 201^{+} Operating Manual

2.7.8. Tick count

Half-body tick visual assessment/counting was performed monthly on the body of the animals and depending on the number found were graded into either 0 or A or B or C.

Zero (0) - no tick found on the body

A (low or mild) - number of ticks between 1 and 10

B (medium or moderate) - number of ticks were between 11 and 50

C (high or severe) - number of ticks was overwhelming (over 50).

They were also speciated either into *Amblyomma spp, Boophilus spp* or *Rhipicephalus spp*. The tick counts were done at the level of identifying them to genus level rather than to species level because we were interested in just seeing the effect of deltamethrin on the number of ticks (see chapter 6).

Particular attention was also paid to checking the inside of the ear and the anal area for *Rhipicephalus spp*. To inspect the animals they were cast down and restrained with ropes in lateral recumbency. In this way ticks that were on the bellies were clearly seen.

2.8. Sample collection in the field

2.8.1. Thick and thin smear preparation

Animals were properly restrained with ropes and blood drawn from the peripheral ear vein by capillary suction pressure using heparinized capillaries after the vein was pierced by sterile lancets. The thick and thin smears were made on the same slide in the field on the sampling sites, dried away from direct sunlight and packed in the slide boxes for fixing and staining later in the field laboratory. The slides were labelled with animal number/name, date of sampling, village name and name of the animal owner. Slides that were frosted at one end were used as this made it easier to label them by writing on the frosted part of the slide using a pencil.

Thick smears were made to speed and efficiently detect the parasites in very low parasitemic animals like those that have mild or chronic infection. This is because the parasites are concentrated in one area. Thin smears are good for identifying the species of the trypanosomes. Thin smears are one cell thick only and this makes it easier to see the structure of e.g. kinetoplast, undulating membrane, free flagellum of the parasites that are used for identification (Boyt, 1986).

2.8.2. Blood spot preparation

Blood for collection on the FTA® cards (Whatman International LTD, UK) was collected from the jugular vein of the animals using 5ml heparin vacutainer tubes (Greiner Bio-one, USA) and 16 gauge needle (Greiner Bio-one, USA). Blood in the vacutainer tubes were transported from the field to the laboratory in the cool box. In the field the tubes were as usual labelled with all the necessary details for easy identification of each animal. The blood spots were later on prepared on the FTA® cards in the laboratory upon arrival from the field (Figure 2.4). Blood in the vacutainer tubes was shaken to ensure homogenous solution before application on the FTA® cards (Whatman International LTD, UK). When applying the blood spots the team made sure that the cards were not over saturated with blood. After applying the blood the cards were dried overnight and packed in special storage bags with desiccant and stored in a cool place for future use.

FTA® Cards is a safe, secure and reliable method for the collecting, transporting, and safe room temperature storaging of DNA. When samples are applied to FTA Cards, the cells are lysed and the nucleic acids are immobilized and stabilized within the FTA Card's matrix. Genomic DNA stored on FTA Cards at room temperature for over 11 years exhibits no loss in PCR efficiency (proven). Expected storage length is over 50 years. FTA® is a chemical treatment, which allows for the rapid isolation of pure DNA. When samples are applied to FTA®-treated paper, cell lysis occurs and high molecular weight DNA is immobilized within the matrix. Amplification or restriction enzyme digestion can be performed directly on the treated paper without the need for extensive extraction procedures. FTA Cards rapidly inactivate organisms including blood borne pathogens and prevent the growth of bacteria and other microorganisms. Samples collected on FTA Cards are so safe they can be shipped via regular postal service without hazardous labelling (DNA testing Centre, ®Inc).

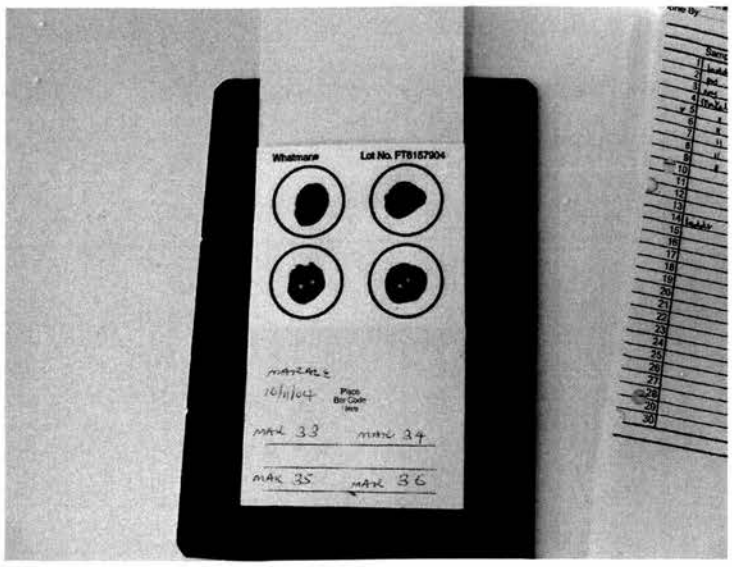


Figure 2. 4 Blood spot on FTA® cards showing how they were labelled in the field.

2.8.3. Data entry and storage

Data in the field were entered on field record sheets (Table 2.1), and later on entered and stored on the computer Excel programme for analysis.

2.9. Laboratory methods

2.9.1. Thick and thin smears examinations

In the laboratory the thin smears were fixed with methanol for 3 minutes and thick smears haemolysed by dipping in water for a few seconds and up to a maximum of 2 minutes (Murry *et al,* 1983). They were then dried and stained with 10% giemsa stain for 30 minutes (Paris *et al*, 1982). After staining the slides were dried and later examined using a 10x100 and 10x50 magnification microscope. The whole smears on the slides were examined to increase the sensitivity and an x50 oil immersion objective lens was used to speed up the examination.

The smears were examined for tsetse-borne disease (trypanosomes) and tick-borne diseases parasites (Theileria, Babesia and Anaplasma). All these parasites can be identified on thick and thin smears that are stained with Giemsa stain. The morphology of these parasites are as described and shown under general introduction and literature review in Chapter one (Figures 1.1, 1.2, 1.9 - 1.13).

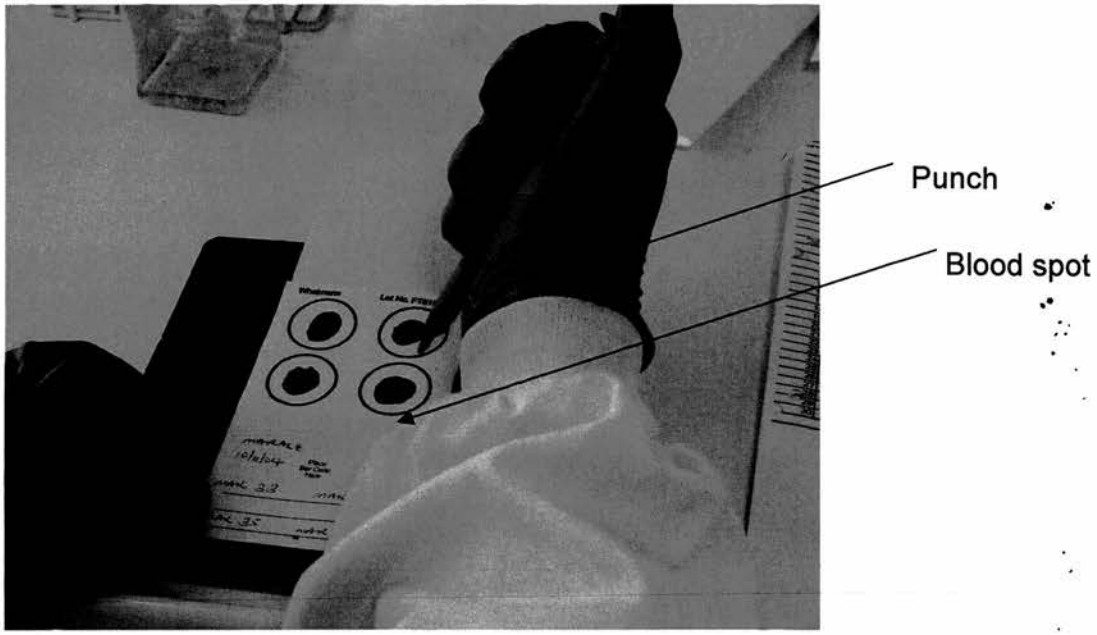


Figure 2. 5 Punching of the blood spot from the FTA® cards.

2.9.2. Internal Transcribed Spacer PCR reactions

2.9.2.1. Preparation (washing) of blood spot for PCR reaction.

In the laboratory using a 2mm diameter punch (Premier, Swiss made), 1 disc was cut from each blood spot sample and dispensed into clean PCR tubes (Alpha Laboratories, UK). The punch (Figure 2.5) was cleaned between samples by cutting two discs from a clean filter paper. A clean disc was also used as negative control. The discs were then washed twice in 200µl of FTA purification reagent (Whatman® International Ltd, UK) for 15 minutes followed by two washes in 200µl of x1 concentrated Tris-EDTA (TE) (10mM Tris HCl pH8; 1mM EDTA) (Sigma-Aldrich Company, UK) for 5 minutes. To minimise contamination between and during washes, one fine-tip plastic pipette (Alpha Laboratories, UK) was used to remove the FTA wash from each PCR tube. Pipettes were then racked in sample order for re-use during all the 4 washes. After the final washing step, discs were transferred into clean PCR tubes, one sample per tube, using clean filter tips (Star Lab GMBH, USA). The discs were then dried for a minimum period of one hour at room temperature prior to amplification.

2.9.2.2. ITS PCR reaction

The PCR was carried out using a nested ITS-PCR method as two separate consecutive reactions (Cox, *et al*, 2005). The ITS-PCR nested PCR depends on amplification of the Internal Transcribed Spacers of the ribosomal RNA gene, of which there are 100-200 copies per trypanosomes genome (Desquesnes, *et al.*, 2001). This PCR amplifies all African animal trypanosomes that include *T. brucei*, *T. congolense* (Forest, Kilifi, Savannah and Tsavo), *T. simiae*, and *T. theileri* and *T. vivax* (Cox, *et al*, 2005), amplicon differ in size due to species specific inter and intra variation in this region.

First round reaction

Each PCR reaction was seeded with a single FTA® disc prepared as described in section 2.8.2.1. Each batch at amplification contained both a positive and negative control.

The 25µl reaction volume contained the following components, Super Taq PCR buffer (HT biotechnologies, Cambridge) (final concentrations of 10mM Tris-HCl pH 9.0, 1.5mM MgCl2, 50mM KCl, 0.1% TritonX-100, 0.01% (w/v) stabilizer), 0.2 µM of each outer primer ITS1 and ITS2 (MWG Biotec) (Table 2.2), 800µM total dNTP's (Bioline Ltd, London), 1.25 units of clear Biotaq (Bioline Ltd, London).

The reaction conditions for the first round were as follows; 1 cycle of 95°C for seven minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes and final extension step for 7 minutes at 72°C and finally stored at 4°C. The thermal cycling was carried out in a DNA Engine DYAD^{TM} Peltier Thermal Cycler (BIORAD, USA).

Second round reaction

For the second round reaction 1µl of the product from the first round reaction was added to a fresh tube containing 24µl of reaction mixture as in first round reaction

but the outer primers were replaced with inner primers ITS3 and ITS4 (MWG Biotec) (Table 2.2), The reaction condition was as in the first round.

Table 2. 2 Sequences of the primers used in Internal Transcribed Spacer PCR reaction.

Primers	Sequence 5' → 3'
Outer ITS1 primer	5' –GAT TAC GTC CCT GCC ATT TG-3'
Outer ITS2 primer	5'-TTG TTC GCT ATC GGT CTT CC-3'
Inner primer ITS3	5'-GGA AGC AAA AGT CGT AAC AAG G-3'
Inner primer ITS4	5'TGT TTT CTT TTC CTC CGC TG-3'

Table 2.3 shows expected band sizes for ITS-PCR as predicted from sequences found in the NCBI database and the band sizes found for ITS-PCR from the published work of Desquesnes *et. al.* (2001).

Table 2. 3 Expected band size from NCBI Database

Species	Expected band size from NCBI Database
T. congolense (Forest)	1513bp
T. *congolense* (kilifi)	1422bp
T. congolense (Savannah)	1413bp
T. congolense (Tsavo)	954bp
T. brucei s.l.	1207~1224bp
T. simiae	850bp
T. vivax	611bp
T. theileri	988bp

2.9.3. PCR for theileriosis

2.9.3.1. PCR used

A novel blood spot PCR assay based on species-specific sequences derived from the *T. parva* p104 antigen gene found in the apical complex localized in the rhoptries, the secretory organelles found in the sporozoites was used to detect the parasite DNA in the blood of infected cattle (Iam, *et al*, 1990; Skilton, *et al*, 2002).

2.9.3.2. Elution of DNA from FTA® cards

In the laboratory using a 3mm diameter punch (Harris Uni-Core, India), 3 discs were cut from each blood spot sample and dispensed into clean 1.5 ml tubes (Alpha Laboratories, UK). The punch was cleaned between punches by cutting two discs from a clean filter paper. Clean disc was also used as negative control.

The discs were then washed once for 15 minutes in double processed tissue culture distilled water (SIGMA-Aldrich Company, UK). No FTA washing reagent was used to wash the blood spot filter papers (discs). Washing with this reagent resulted into poor PCR products. Unlike trypanosomes that are extra cellular parasites *T. parva* are intracellar parasites i.e. found inside the red blood cells. It seems when we were washing the blood spot filter paper with the FTA reagent much of the *T. parva* DNA was washed off together with the red blood cells prior to amplification.

After washing the discs they were transferred from 1.5 ml tubes into PCR tubes (Alpha Laboratories, UK) and 100µl of double processed tissue culture distilled water added to them. They were then heated for 30 minutes at 95°C for elution. After heating, elute of each sample was used for PCR reaction.

2.9.3.3. p104 gene PCR reactions

From the elute 3µl was added to 22µl of master mixture. The reaction master mixture for a 25µl consisted of the following: 1x NH_4 reaction buffer ((16mM $(NH_4)_2$ $S0_4$, 67mM Tris-HCl (pH8.8 at 25°C), 0.01% Tween-20) (Bioline Ltd, UK), 50mM $MgCl_2$ solution (final concentration 1.5mM) (Bioline Ltd, UK), 0.4µM of each p104 nested forward (internal) primer and) p104 nested reverse (internal) primer (MWG Biotec), 800µM total dNTP's (Bioline Ltd, London), 1U of Red Taq (Bioline Ltd,

UK). Each PCR reaction was run with a positive control (liquid genomic DNA) and negative control (elute from clean blank FTA disc).

Table 2 .4 Sequences of the primers used to amplify p104 gene of *Theileria parva*.

Primers	Sequence 5' → 3'
p104 nested forward (internal)	5'-GGC CAA GGT CTC CTT CAG AAT ACG-3'
p104 nested reverse (internal)	5'-TGG GTG TGT TTC CTC GTC ATC TGC-3'

Amplification reaction conditions were as follows: 94°C for 3 minutes once to denature the DNA template followed by 35 cycles of 94°C for 45 seconds followed by 55°C for 45 seconds to anneal the primers to the template then by 72°C for 1 minute. This was followed by 72°C for 5 minutes to extend the product and finally stored at 4°C. The thermal cycling was carried out on a Peltier Thermal Cycler (BIORAD, USA).

2.9.4. Agarose Gel Electrophoresis

2.9.4.1. Agarose Gel Electrophoresis – ITS PCR

A 1.5% (w/v) agarose gel was prepared with 1x concentrated Tris Borate EDTA Buffer (TBE buffer, 89mM, Tris Borate, pH approximately 8.3, containing 2mM EDTA) (Sigma-Aldrich, poole, Dorset, UK) stained with 6.25µM ethidium bromide. Once the second round was finished, 5µl of gel loading buffer (SIGMA-Aldrich Company, UK) was added to the reaction mixture and 15µl loaded onto the 1.5% (w/v) agarose (Bioline Ltd, London). A 100bp (12.5µl) graduation molecular maker (Abgene 1000bp MID), positive control (15µl) and negative controls (15µl) were run alongside the samples. Agarose gel electrophoresis was carried out with a 30cm by 20cm run at 100 volts for about 45 minutes to one hour. Amplification products were visualized using a transluminator, Gel Doc 2000 (BIORAD, USA).

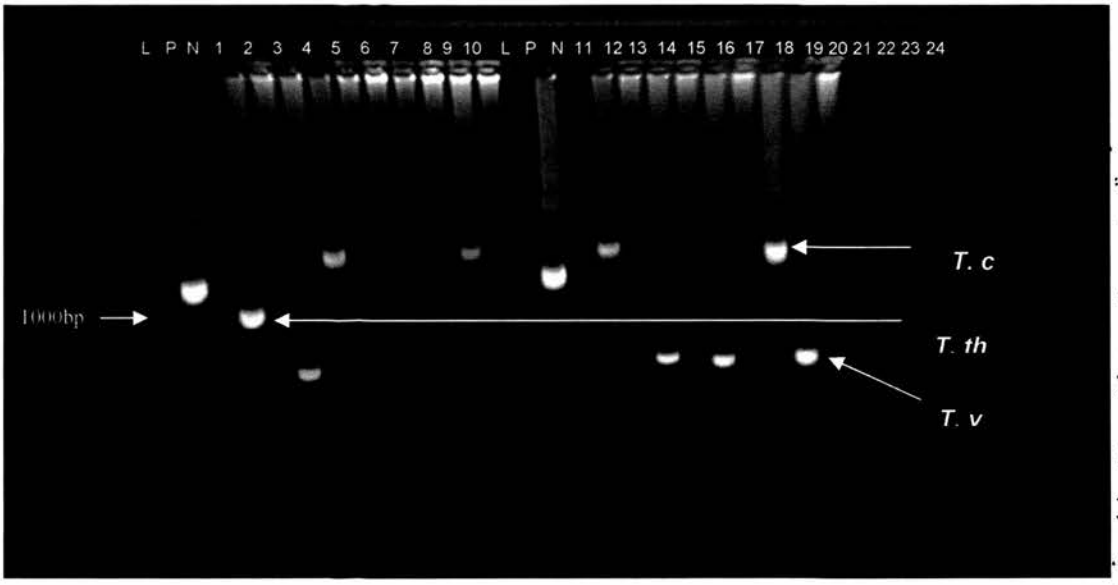


Figure 2. 6 PCR bands of trypanosomes from Eastern province Zambia L=maker (100bp ladder), P (Positive control = *T. brucei*), N (Negative control) and Lines 1-19 are field samples.

2.9.4.2. Agarose Gel Electrophoresis – p104 PCR

The procedure was conducted as above (section 2.8.4.1). The *p*104 gene PCR (Iams, *et al*, 1990, Skilton, *et al*, 2002) generated a band size of about 278bp (Figure2.7).

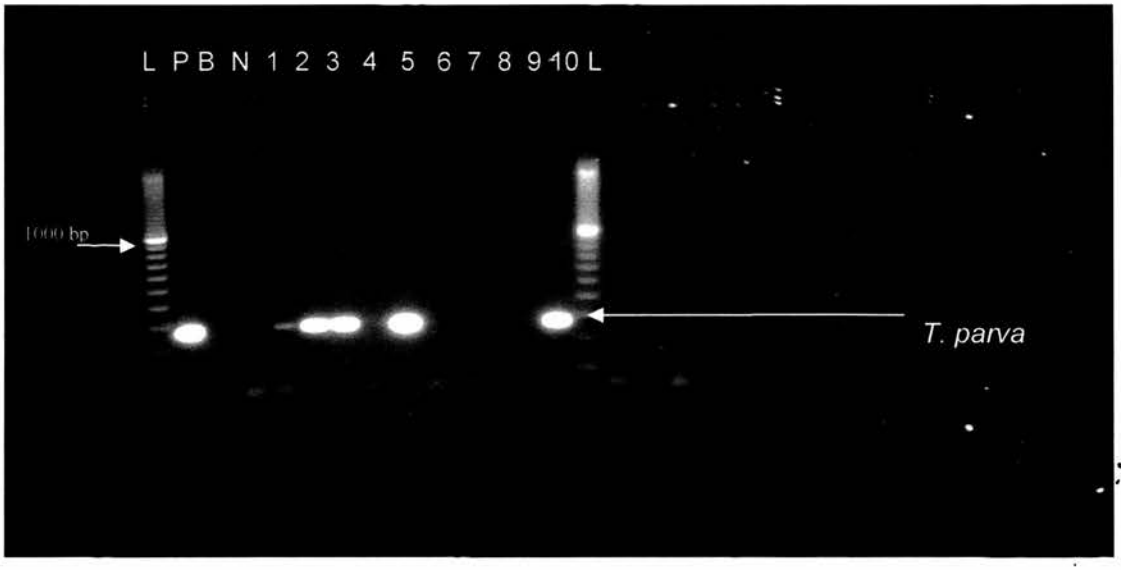


Figure 2. 7 PCR bands of *Theileria parva* from Eastern Province of Zambia. L=marker (100bp ladder), P (Positive control = *T.parva* marikebuni), B (Blank), N (Negative control) and Lines 1-10 are field samples from Petauke District, Zambia.

2.10. Data Analysis

Data on trypanosome and tick-borne infections was compiled, entered and stored in Microsoft Excel spreadsheet for analysis. Households demographic characteristics and livestock herd structure for Mambwe District were also entered on Microsoft Excel spreadsheet for analysis. Calculation of summary descriptive statistics was done using Minitab 13.2 software. Graphs were done using Microsoft Excel worksheets and Minitab 13.2 software. Statistical comparisons by proportion was done by Chi-square tests using Minitab 13.2 software packages and when the sample sizes were small and expected values were less than 5, comparisons of the proportion was done by Fisher's exact test using R. version 2.4.1 software package. Win Episcope 2.0 programme was used to determine the Kappa values for the agreement between tests that were used for trypanosomiasis and theileriosis diagnosis. Survival analysis using R version 2.4.1 software package was used to determine period of protection offered by isometamidium chloride that were reported to have been treated by the farmers and Veterinary Officers in Mambwe District. Survival analysis using R version 2.4.1 software package was also used to determine the time animals survived infection under different treatment regimes in Petauke District.

3. CHAPTER THREE

3 HOUSEHOLDS DEMOGRAPHIC CHARACTERISTICS AND LIVESTOCK HERD STRUCTURE IN MAMBWE DISTRICT.

3.1. Introduction

The objectives of this chapter were to study the demographic characteristics and livestock herd structure in recently-settled area in Mambwe District. Census of livestock and households in the study area in Mambwe District were needed in order to identify the households to be interviewed and the sample size of the animals to be sampled during the cross-sectional study that was conducted in the area. This was achieved by conducting animal and household census in the study area in Mambwe District from the base of the valley to the plateau in a transect manner (Figures 3.1a and 3.1b; Appendix 7a and 7b).

Household demographic characteristics and livestock herd structure were determined by the use of a questionnaire. The questionnaire, contained sections on human and livestock demography, livestock management, crops cultivated and mosquito control. One section gave some details on cotton because it is the most important cash crop grown in the area and the pesticides used often contain chemicals (deltamethrin) that have an effect on tsetse flies (Thompson *et al*, 1991). Deltamethrin also has effect on mosquitoes (Sharma *et al*, 2005) including the vectors of malaria and ticks (Chizyuka and Luguru, 1986; Okello-Onen *et al*, 1994) the vectors of tick-borne diseases.

It is hypothesised that in the last few years there has been a high emigration of farmers and their livestock from the plateau into the valley. The main occupation of these farmers is hypothesised to be farming mainly cotton growing.

3.2. Materials and methods

3.2.1. Study area

Mambwe District.

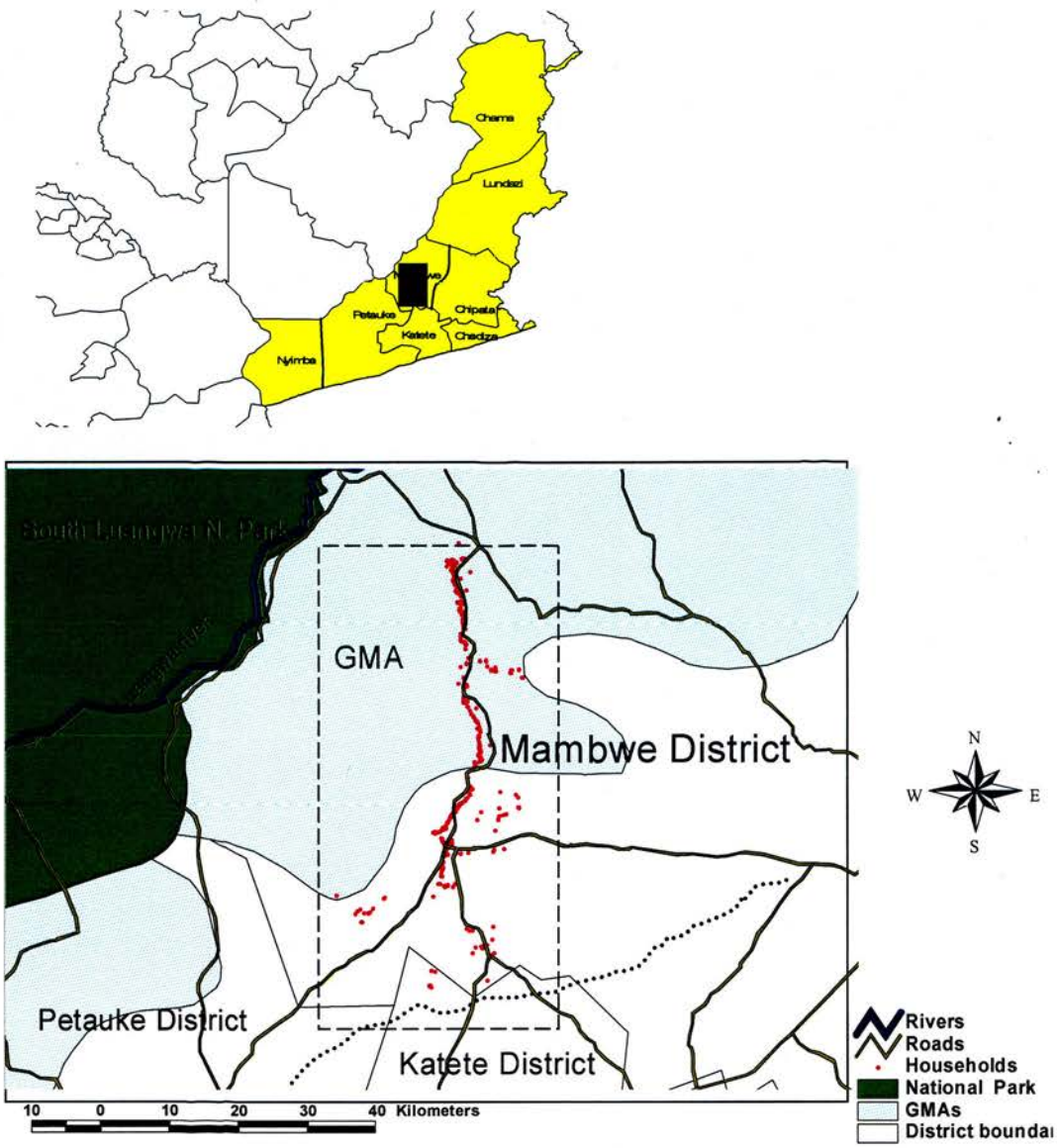
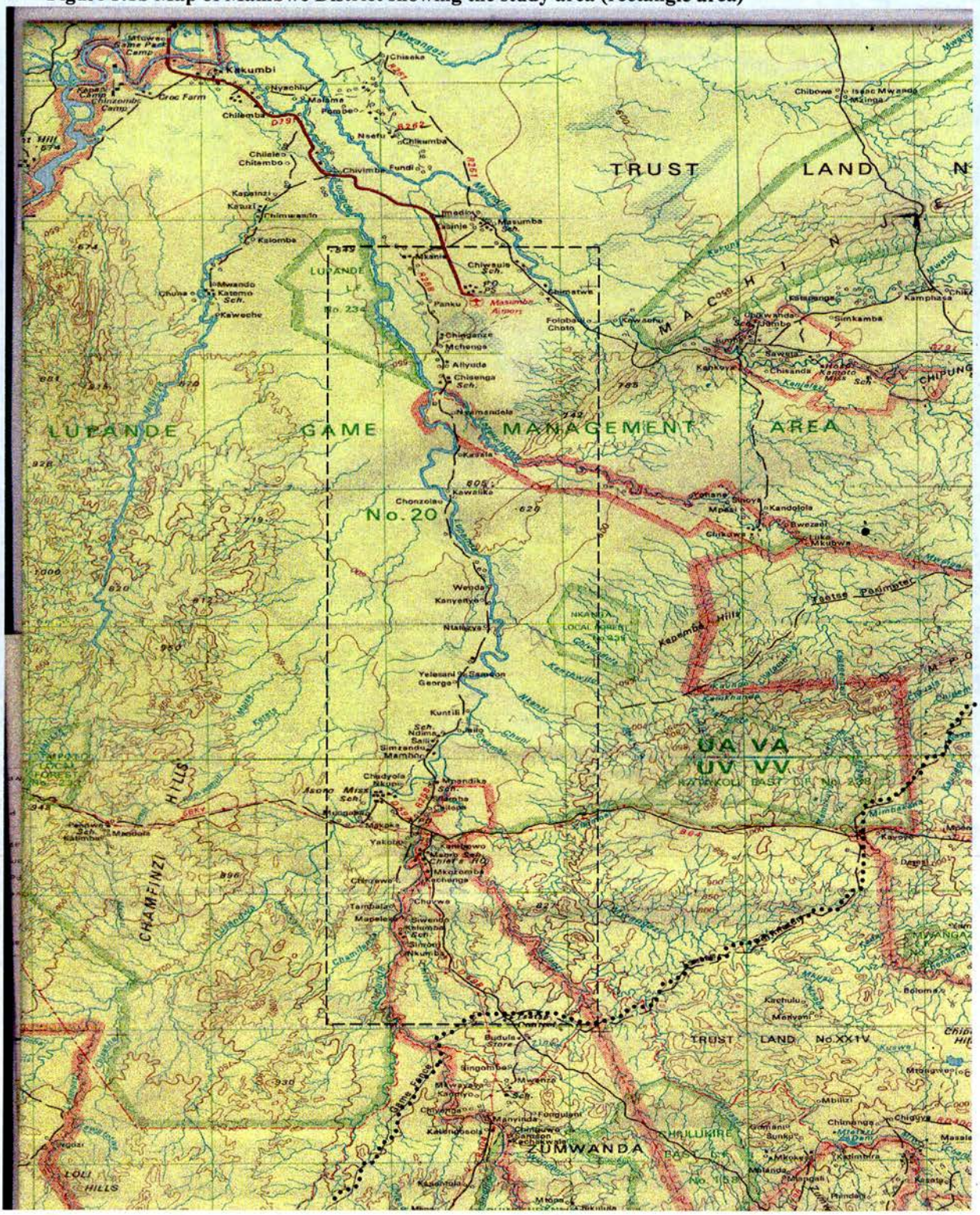


Figure 3.1a Map of Mambwe District in Eastern Province showing the area (indicated by a rectangular area) where a cross-sectional survey was conducted. Insert above is a Map of Zambia showing the location of Eastern Province. The Black dotted line (.....) shows where the old game fence passed.

Figure 3.1b Map of Mambwe District showing the study area (rectangle area)


TRUST LAND
LUANGWA
LUPANDE GAME MANAGEMENT AREA
No. 20
LUPANDE
No. 234
CHAMFINZI HILLS
LOLI HILLS
UMWANDA
TRUST LAND No. XX1V
Kakumbi
Chilemba
Kapainzi
Kaloma
Kaweche
Chiwaula Sch.
Panku
Chiengaze
Mchenga
Allyuda
Chisenga Sch.
Nsemandele
Kasala
Kawaika
Chonzolau
Wenda
Kanyenya
Ntalaya
Yelesani
Kuntili
Simzandu
Mambwe
Chadyola
Nkupu
Mpandika Sch.
Mkokomba
Kachenga
Chiruwe
Tambalale
Mapalesa
Sikemba
Kalumba Sch.
Simoni
Nkumba
Budula Store
Singombe
Mwenze
Kanyiyo
Chiwanga
Chinsen
Kachinjoloso
Kasongole
Mtapa
Nkunda
Chiwanga
Forgulani
Manyinda
Chimenga
Mkokwe
Malanda
Mpangali
Kasaka
Mpanda
Chibowe
Isaac Mwanda
Mzinga
Kapamba Hills
Kandolola
Mpezeni
Chikowa
Vikubwa
Kamphasa
Simkamba
Kawesefu
Kankoya
Chisanda
Kamoto
Mpika Sch.
Chipungu
Kanjelesi
Mwangazi
Chiseka
Nyachilu
Malama
Pombe
Nseefu
Chilumba
Chilelea
Chitembo
Chivimba
Fundi
Kapainzi
Kasuzi
Chimwanda
Kalomba
Imadiki
Kasanje
Masumba Sch.
Mkanie
Chiwaula Sch.
Chimatwa
Folobala
Chato
Kawesefu
Mwando
Katemo Sch.
Chuma
Kaweche
Mwangazi
Luangwa
Lupande
Mwaya
Kapamba Hills
Tentsa Pedimtep

3.2.2. Household and Livestock Census

A household and livestock census was conducted by visiting all the households in the study area. All the houses visited (Figure 3.1a and 3b; Appendix 7a and 7b) during this "household to household" census were geo-referenced using the global positioning system (GPS) for map production. This also made it subsequently easier to re-locate households to administer more detailed questionnaires to determine household demographic characteristics and livestock herd structure (Section 3.2.4). Households were also easily re-located during the animal trypanosomiasis survey that was conducted immediately after the census (Chapter 4). The census was conducted by using two motor bikes and two 4X4 vehicles. To maintain consistency in the data collected standardised field record sheets were used by all the enumerators during the census. All the enumerators knew the local language used in the area (see below) and were trained on how to fill in the field data sheets. Two handheld units GPS were used to geo-reference all the households in the area. One GPS was used by the author and the other one by census enumerators who were trained on how to use them before going in the field. The field data sheet was divided into two sections. The first section covered the human census and the second section was on the livestock census. The field data sheets are shown in Appendix 3.

The census was conducted in May 2005; this was followed by a structured questionnaire administered in September 2005 and a survey of animal trypanosomiasis conducted in the same area.

3.2.3. Structured Household survey questionnaire

The interviews were conducted in one of the local languages (Nyanja/Chewa) used in the area. The local enumerators who conducted the interviews were trained both in a classroom situation and by administering the questionnaire in the field. Care was taken that all avoided biasing the results with their own expectations or beliefs, or influencing the respondents in any way. The researcher (the author) supervised and worked closely with them to ensure this throughout the survey.

Primary data were collected using a standardised questionnaire (see Appendix 4). The questionnaire was divided into five sections, which included information on the household head (Section one), household relocation i.e. when the household moved into the area (Section two), crops grown in the area (Section three), malaria control (section four) and livestock management and disease control (Section five). Leading questions were avoided in the questionnaire to avoid getting bias in answers from the farmers.

3.2.4. Selection of households

Selection of households for interview was based on household and livestock census conducted in the area. The selection of the households considered the combination of livestock species kept by a household (Table 3.1, Figures 3.2). Interviews were conducted on "household to household" basis. In the area there were 980 households. All households (272 households) with any cattle, small ruminants or pigs (in the categories 2 – 8, Table 3.1, Figure 3.2) were interviewed. A proportion of households (227 households) from category 1 with none of these species, selected at random were also interviewed.

Table 3. 1 Combinations of livestock kept in households in the study area.

Category	Combination of Livestock	Cattle	Goats	Sheep	Pigs	Number of households
1	None	0	0	0	0	708
2	Cattle only	365	0	0	0	66
3	Small ruminants only	0	705	8	0	104
4	Pigs only	0	0	0	118	25
5	Cattle and small ruminants	235	394	38	0	41
6	Cattle and pigs	37	0	0	34	8
7	Small ruminants and pigs	0	128	0	67	16
8	Cattle, small ruminants and pigs	48	123	0	43	12
	Total	685	1350	46	262	980

A total of 453 households keep only chickens (4493); these are included in category 1

- A total of **499 households** were selected for interview from the 980 households in the area. The breakdown of the **499** households is as indicated below.

- This was calculated on the basis of 95% level of confidence with a desired precision of 5% and estimated proportion of 50% of households. The calculated sample size (384) was also adjusted for the estimated sample design effect (1.3 x 384 = **499**) (Aday, 1996).

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$$

n = sample size

p = estimated proportion

d = desired precision

$Z_{1-\alpha/2}$ = Standard error associated with confidence interval (95%CI = 1.96)

- All 272 households with any ungulates (cattle, small ruminants, pigs) see figure 3.2 (A,B and C) were interviewed
- Out of the 708 households without ungulate livestock (453 households keeping chickens only and 255 households without any livestock), 227 households were interviewed. The number 227 of HH was calculated as follows; 499 (total HH interviewed) – 272 (HH in A, B and C).

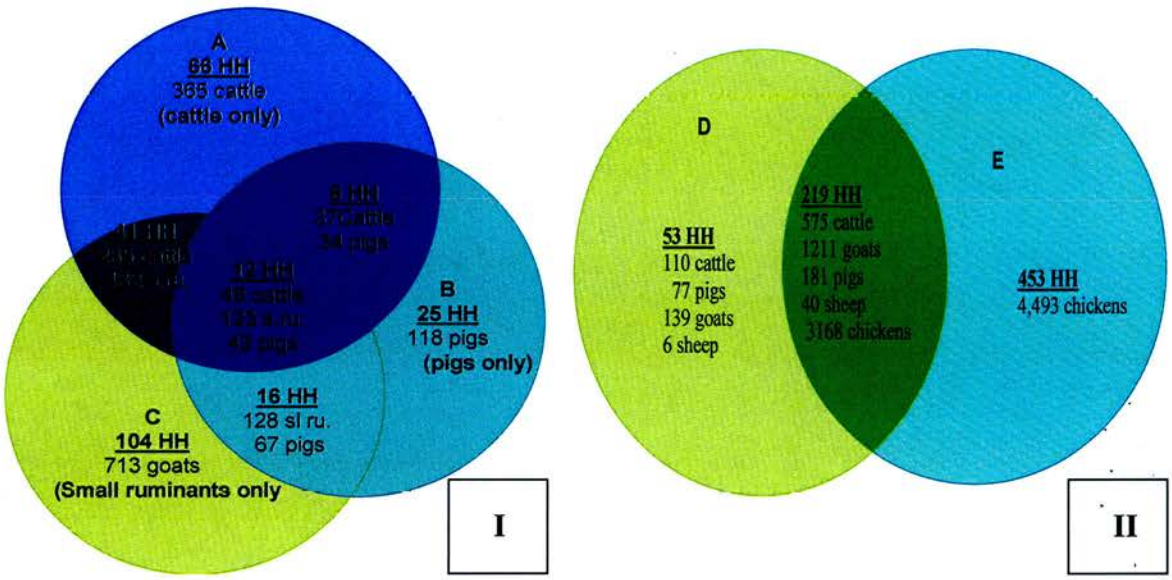


Figure 3. 2 Combinations of livestock kept, number of households and number of livestock. S. ru = small ruminants (goats and sheep). HH = households

I. Combinations of ungulates **II. Combinations of mammalian and avian livestock**

3.3. Results

3.3.1. Household and Livestock Census

A total of 980 households were included in the census. Out of this number, 793 (80.9%) are headed by men and only 187 (19.1%) households are headed by women. Significantly more households were headed by men than women (χ^2 = 722, 95% d.f., $p < 0.001$). The mean number of people per household in the study area was 8 persons (median: 7, range: 1 – 40) per household (Table 3.2)

Table 3. 2 Human population in the study area (Mambwe).

	Men	Women	Boys ≤15yrs	Girls ≤15yrs	Total	Average people/Household
People slept in the house today (*de facto* population)	1958	2044	2017	1962	7982	**8.1**
People in the households (*de jure* population)	**2034**	**2103**	**2044**	**1994**	**8175**	**8.3**

***de facto* population:** This refers to the usual household members present and visitors who spent the census night at any given household.

***de jure* population:** This refers to usual household members present and usual household members temporarily absent at the time of the census.

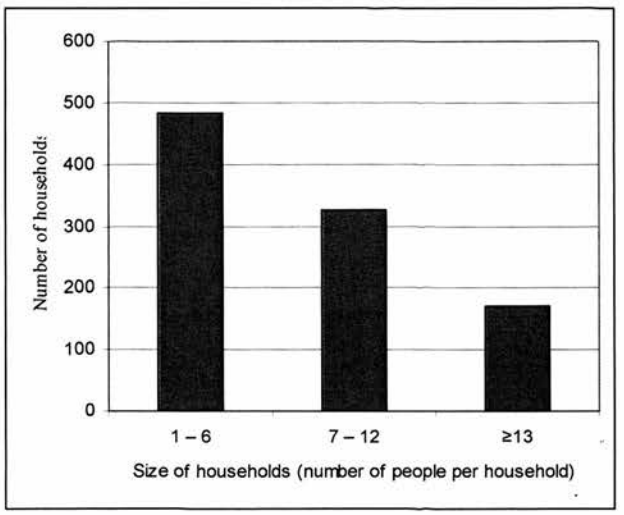
For all classes de *facto* population was smaller than de *jure* population because most of the members of the households were not in the area at the time of census. Both de *facto* population and de *jure* population were done to see the movement of the member of the households.

There were more households with small family sizes (484 = 49.4%) than with medium size (325 = 33.2%) and this was highly significant (χ^2 = 52.5, d.f. = 1, $p < 0.001$). Households with small family sizes were also more when compared with large family sizes (171 = 17.4%) and was statistically significant (χ^2 = 223.2, d.f. = 1, $p < 0.001$). See Table 3.3 and Figure 3.3 for the definition of small, medium and large family sizes.

Table 3.3 Family size (Mambwe).

Family size	Label	Households
1 – 6	Small	484
7 – 12	Medium	325
≥13	Large	171
	Total	**980**

Figure 3.3 Family size (Mambwe)



The number of households keeping a combination of either two, three or more of the livestock types (cattle, goats, sheep and pigs) are tabulated below (Table 3.4 and Figure 3.4). There were 66 households (6.7%) that kept cattle only, 104 households (10.6%) kept only small ruminants, 25 households (2.6%) kept only pigs, 41households (4.2%) kept a combination of cattle and small ruminants, 8 households (0.8%) kept a combination of cattle and pigs. A combination of small ruminant and pigs was kept by 16 households (1.6%) and 12 households (1.2%) kept a combination of cattle, small ruminant and pigs. A total of 708 households (72.2%) did not keep any of the above livestock type (Appendix 7b).

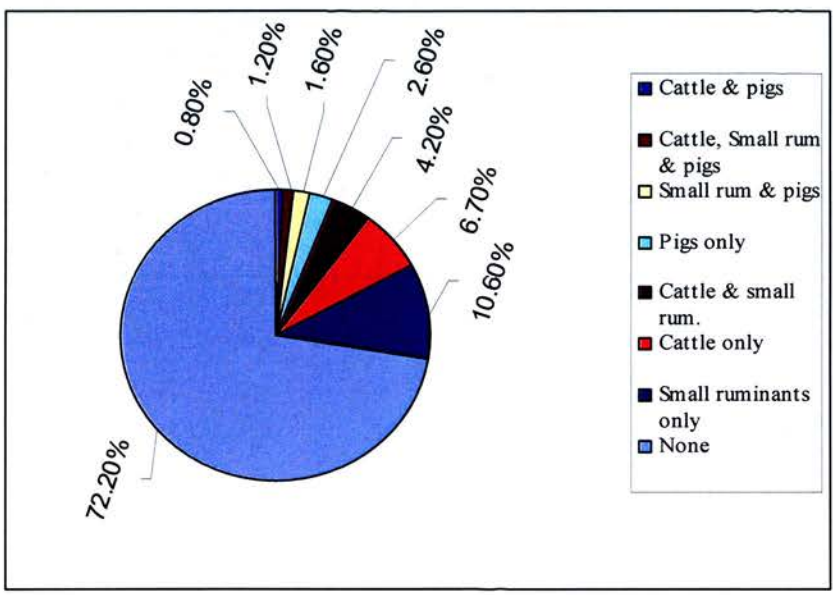
More households (104) kept only small ruminants (goats and sheep) than the number keeping any other combination of livestock. This was highly significant ($\chi^2 = 197.5$, d.f. = 6, $p < 0.001$)

Table 3. 4 Livestock kept and the combination in Mambwe district.

	Livestock combination	Cattle	Goats	Sheep	Pigs	Number of HH	HH (%)
1	None	0	0	0	0	708	72.2
2	Cattle only	365	0	0	0	66	6.7
3	Small ruminants only	0	705	8	0	104	10.6
4	Pigs only	0	0	0	118	25	2.6
5	Cattle & small rum.	235	394	38	0	41	4.2
6	Cattle & pigs	37	0	0	34	8	0.8
7	Small rum & pigs	0	128	N/A	67	16	1.6
8	Cattle, Small rum & pigs	48	123	N/A	43	12	1.2
	Total	685	1350	46	262	980	100

rum = ruminant HH =households

Figure 3. 4 Livestock kept and the combination in Mambwe district.



3.3.2. Livestock kept in the area

All cattle kept in the area were local breeds (mainly Angoni). The number of livestock kept in the area at the time the census was conducted in May 2005 included cattle (685), goats (1350), sheep (46) and pigs (262) (Appendix 7b). All the goats

(valley goats), sheep (thin-tailed) and pigs (Nsenga strain) were local breeds. The mean number of cattle was 0.7/head per household (Table 3.5). The combinations of either two, three or more of the livestock types (cattle, goats, sheep and pigs) kept in the area are shown in Table 3.4 and Figure 3.4 above. Other types of livestock kept were chickens (7661), ducks (385), doves (814), guinea fowls (133), dogs (835) and cats (76).

Excluding chickens (7661) and doves (814) there were more goats (1350) kept in the area than any other type of livestock. Very few sheep (46) were kept in the area at the time the census was conducted.

Cattle were classified into male and female and further into calves (still suckling = 57), weaners (those that had stopped suckling = 62), and adult bulls (68), draft oxen (334) and cows (dry and milking = 149). There were more draft oxen than any other cattle in the other 4 categories (Table 3.6). This was statistically significant (χ^2 = 516.70, d.f. = 4, p-value < 0.001). In the cow category there were more dry cows (97) than milking ones (52) and this was statistically significant (χ^2= 25.99, d.f.= 1, p < 0.001). Table 3.5 shows the minimum, maximum and average number of each livestock type by households.

Table 3. 5 Livestock kept in the study area, Mambwe district.

	Cattle	Goats	sheep	Pigs	Chickens
Total	685	1350	46	262	7661
Mean	0.70	1.38	0.05	0.27	7.82
Min	0	0	0	0	0
Max	32	65	15	12	71
StDev	2.7	4.8	0.6	1.2	10.8

Table 3. 6 Classification of cattle kept in the area

	Male cattle				Female Cattle			
	Suck-ling ♂	weaned ♂ ≤2	Bulls	Draft oxen	Suck-ling ♀	Weaned ♀ ≤ 2	Dry cows	Milking cows
	26	**32**	**68**	**339**	**31**	**40**	**97**	**52**
Mean	0.03	0.03	0.07	0.35	0.03	0.04	0.10	0.05
Min	0	0	0	0	0	0	0	0
Max	3	8	8	10	3	5	11	6
StDev	0.22	0.32	0.44	1.17	0.23	0.26	0.61	0.36

♂= male, ♀= female

3.3.3. Households survey

A total of 501 households out of the total households of 980 in the study area were interviewed using the structured questionnaire. The breakdown of the households interviewed in each category is shown below (Table 3.7). In each category the coverage of the households successfully interviewed out of the number intended was over 87% (mean: 95.1% range 87.5% to 100%). The coverage in each livestock keeping category was; category 1 (227/227: 100%), category 2 (60/66: 90.9%), category 3 (93/104: 89.4%), category 4 (25/25: 100%), category 5 (38/41: 92.7%), category 6 (8/8: 100%), category 7 (14/16: 87.5%) and category 8 (12/12: 100%). In category 1, in addition to the 227 households three households insisted to be interviewed (Table 3.7).

Table 3. 7 Number of households interviewed in each category.

Category	Combination of livestock	HH in the area	Expected HH to be interviewed	HH interviewed	% HH interviewed
1	None	708	227	251	100
2	Cattle only	66	66	60	90.9
3	Small ruminants only	104	104	93	89.4
4	Pigs only	25	25	25	100
5	Cattle and small ruminants	41	41	38	92.7
6	Cattle and pigs	8	8	8	100
7	Small ruminants and pigs	16	16	14	87.5
8	Cattle, small ruminants and pigs	12	12	12	100
Total		**980**	**499**	**501**	

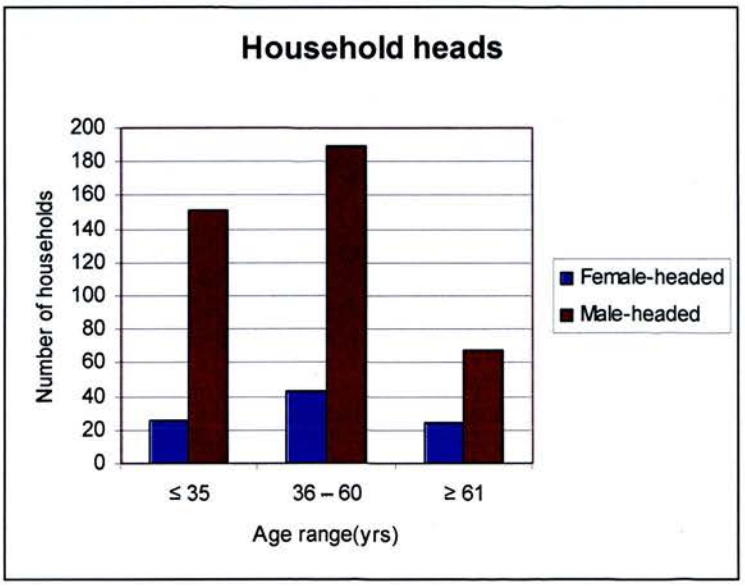
3.3.4. Age and sex of household heads

Most of the heads of the households were male (408/501; 81.4%). Female-headed households were only 18.7% (93/501). Most households were headed by males, aged 36-60 years (189; 37.7% of total HH interviewed). Males 35 years or less were the next most frequently category of household head (151; 30.1% of HH) followed by males greater than 60 years old (68; 13.6% of HH). See Table 3.8 and Figure 3.5 below.

Table 3. 8 Male and Female household heads

Age range (yrs)	Number of female	%	Number of male	%	Total
≤ 35	26	14.7	151	85.3	177
36 – 60	43	18.5	189	81.5	232
≥ 61	24	26.1	68	73.9	92
Total	**93**	**18.6**	**408**	**81.4**	**501**

Figure 3. 5 Male and Female household heads



3.3.5. Household relocation (migration)

Out of the 501 respondents, 244 (48.7%) were immigrants and 257 (51.3%) were born in the area; the difference was not significant ($\chi^2 = 0.575$, d.f. = 1, p = 0.448). Of the households that were immigrants, the majority of them (121/ 244: 49.6%) moved into the area within the last five years. The number of people coming into the

area started increasing 16-20 years ago (Table 3.9 and Figure 3.6). Eighty percent (80%) of immigration occurred within the last 10 years.

Figure 3. 6. Households relocation (immigration)

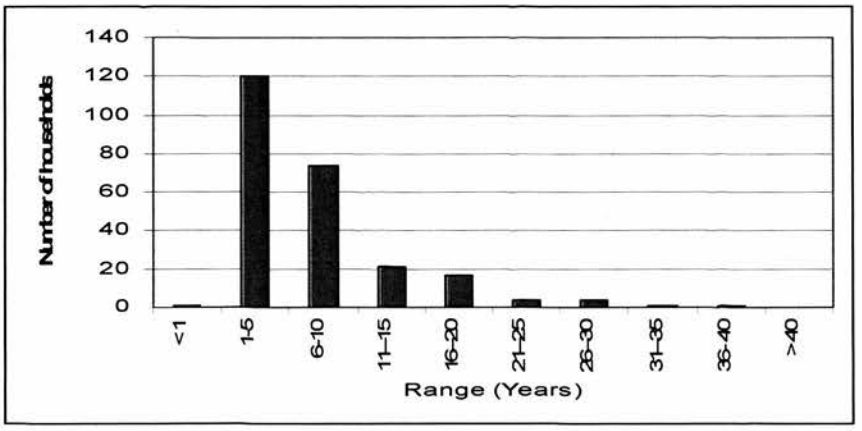


Table 3. 9 Year households immigrated in the area.

Year moved (yrs)	HH headed by Men	HH headed by Women	Total	%
Born in the area	191	66	257	51.3
< 1	1	0	1	0.2
1-5	108	12	**120**	24.0
6-10	69	5	**74**	14.8
11–15	20	1	21	4.2
16–20	12	5	17	3.4
21–25	3	1	4	0.8
26–30	3	1	4	0.8
31–35	0	1	1	0.2
36- 40	0	1	1	0.2
> 40	0	0	0	0
Did not remember	1	0	1	0.2
Total	**408**	**93**	**501**	

3.3.6. Reasons for immigration

The immigrant respondents were asked to give reason why they migrated into the area. The majority of the households 111 (45.5%) said they came from neighbouring districts to the area looking for fertile land for farming. The second major reason of immigration was that of following their parents (35 HH, 14.3%) and the number of households that gave the third reason of that of marriage was 34 HH (13.9%) as a result of some husbands following their wives' and establishing homes. A full list is tabulated (Table 3.10).

Table 3.10 Reasons for immigration.

	Reasons for immigrating	Number of Households	%
1	Looking for farming/fertile land	111	45.49
2	Followed parents	35	14.34
3	Marriage	34	13.93
4	Retirement	26	10.66
5	Employment	14	5.74
6	Family dispute	8	3.28
7	Followed relatives	8	3.28
8	Death of father/relative	4	1.64
9	Original place was water-logged	2	0.82
10	Tradition leadership (village Establishment)	1	0.41
11	For school	1	0.41
	Total	**244**	

3.3.7. Source of income and earnings

The main source of income in the area was farming (Table 3.11). About 84.0% (421) of the households interviewed depend on farming for their income. The difference between the number of the households that depended on farming as their source of income and the number of households (80) that depended on other sources of income was statistically significant (χ^2= 461.477, df = 1, $p < 0.001$). The average income per household per annum was about ZMK1,849,131 (US$ 377.85). The maximum was

K20,000,000 (US$6,250) and the minimum was K40,000 (US$12.5) per household per annum. The dollar exchange rate at the time the survey was conducted was US$ 1 to ZMK3,200.

Table 3. 11 Source of income

	Source of income	Number of households	%
1	Farming only	421	84.03
2	Employment and farming	11	2.2
3	Employment only	10	2
4	*Temporal work	6	1.2
5	Farming and livestock sales	5	1
6	Farming and business/shop	3	0.6
7	Farming and carpentry	1	0.2
8	Life pension	1	0.2
9	Making clay pots for sale	1	0.2
10	None	42	8.4
		501	

*** Work of not permanent nature**

3.3.8. Land ownership and type of the houses

The average land holding size in the area was 5.86 acres (median: 3.5 acres, range: < 1– 120 acres) per household. The mean land holding allocated for crops was 4.34 acres (median: 3 acres, range: 0-50 acres) and for the livestock grazing 0.58 acres (median: 0, range 0-70 acres) per household (Table 3.12). Houses in this area were built with either mud walls or fired bricks, and roofed with either grass thatched or iron sheeting. Mud walls and grass thatched are indication of poverty; those who could affort to would build with bricks and iron sheets. There were more houses (334) in the area that were made out of mud walls and grass thatched roofs than from other type of material (167). See Table 3.13.

Table 3.12 Land owned

Average land size (Acres) per HH	Average acreage per HH allocated to livestock for grazing	%	Average acreage allocated to subsistence cropping per HH	%
5.86	0.58	9.9	4.34	74.1

Table 3.13 Type of houses in the area, in order of increasing cost

	Type oh house	Number of households	%
1	Mud walls and grass thatched roof	334	66.67
2	Mud walls and iron sheet roof	30	5.99
3	Burnt brick walls and grass thatched roof	51	10.18
4	Burnt brick walls and iron sheet roof	86	17.17

3.3.9. Crops, fertilizer and manure usage

Maize, cotton and groundnuts were the crops most commonly grown in the area (Table 3.14). Four hundred and fifty eight (91.4%) households grew maize, 398 (79.4%) households grew cotton and 150 (29.9%) households grew groundnuts (Table 3.14). Cotton was cultivated as a cash crop and the other crops were mainly for home consumption. Only 6 (1.2%) households used fertilizer while 53 (10.6%) households used livestock manure mainly from goats and cattle. During the growing season some of the farmers move from their permanent homes to temporary shelter close to the fields to chase away wild animals that destroy and eat crops. The number of households that reported doing so was 113 (22.6%).

Cotton seed and pesticides are provided to the farmers on loan by two companies on a contract basis (Table 3.16). The loan is repaid after they harvest and sell their cotton produce. Farmers are not allowed to sell cotton to people other than the companies that supplied the farm inputs. The companies come to collect the cotton after harvesting.

Since most of the farmers find it difficult to determine the distance from their houses to their cotton fields, the distance was judged in terms of minutes/hours it takes them to walk from the houses to the cotton fields. On average it takes the farmers 30 minutes to walk to the fields (Table 3.15).

Table 3. 14 Crops grown in the area

Crops grown	Number of HH growing crop	Percentage (%) of HH growing crop
Maize	458	91.4
Cotton	398	79.4
Groundnut	150	29.9
Rice	11	2.2
Sunflower	43	8.6
Sorghum	9	1.8
Soya beans	6	1.2
Tobacco	4	0.8
Cassava	4	0.8
Cowpea	2	0.4
Beans	4	0.8
Vegetables	2	0.4

HH = households, n= 501

Table 3.15 Time takes to walk to the field.

Time takes to walk to field (minutes)		
Mean	**Max**	**Min**
30	180	Within home (<1)

Table 3. 16 Pesticides used by farmers

Name of insecticides	Number of households	Source (company)
Deltamethrin	244	Clark Cotton
Cypermethrin	174	Dunarvant

3.3.10. Mosquito control and number of cases

Out of the 501 households interviewed, 367 (73.3%) use mosquito bed nets and 398 (79.4%) use anti-malaria drugs (Table 3.17). There were more people using anti-malarial drugs compared with those that were using bed nets and this was significant (χ^2 = 4.97, d.f = 1, p = 0.026). On average there were 4 cases of malaria per household per year. The mean distance of households from health centres was 10km (Table 3.18).

Table 3.17 Mosquito bed net and drug used

	Use mosquito bed nets		Use of drugs	
	Yes	No	Yes	No
Number of Households	367 (73.25%)	134 (26.75%)	398 (79.44%)	103 (20.56%)

Table 3.18 Malaria cases and distance of households from Health centres.

	Mean	Max	Min	Mode	Median
Number of cases per annum per household	4	20	0	3	3
Distance from the Health centres (km)	10	30	<1	5	10

3.3.11. Livestock ownership and management

The average time the people had kept cattle was 5 years (median 4 years and range 27 - <1 year). Farmers had been keeping sheep for a mean of 2 years (median 2 years and range 1 – 7 years). The mode was high (4 years) for goats and for the other livestock species it was 2 years (Table 3.19). Over 92 % of the households acquired livestock by buying (Table 3.20). The most important reason given by the respondent (189/383 = 49.4%) for not owning cattle was that of lack of finance. The second important reason (60/383 = 15.7%) was that of tsetse flies (Table 3.21).

Table 3.19 Livestock ownership.

		Length of keeping (yrs)				
Livestock Kept	**Number of HH**	**Mean**	**Max**	**Min**	**Mode**	**Median**
Cattle	117	5.24	27	0.33	2	4
Goats	148	4.24	20	0.33	4	4
Pigs	42	3.52	12	0.33	2	2
Chickens	351	2.89	40	0.08	2	2
Sheep	8	2.5	7	1	2	2

Table 3.20 Source of livestock

Livestock	**Bought**		**Inherited**		**Dowry**		**Loan**		**Given**	
	Number of HH	**%**	**Number of HH**	**%**	**Number of HH**	**%**	**Number of HH**	**%**	**Number of HH**	**%**
Cattle	113	95.76	3	2.24	0	0	3	2.25	0	0
Goats	140	94.59	6	4.05	0	0	0	0	1	0.68
Pigs	39	92.86	3	7.14	0	0	0	0	0	0
Chickens	326	92.88	15	4.27	0	0	0	0	0	0
Sheep	8	100	0	0	0	0	0	0	0	0

Table 3.21 Reasons for not keeping animals.

Reasons for not keeping cattle	Number of HH	Percentage (%)
No Cash	189	49.4
Tsetse problem	60	15.7
Disease	27	7.1
Too old to keep cattle	6	1.6
Cattle not available	2	0.5
Not ready to keep	1	0.3
Just settled in the area	2	0.5
Others	96	25.1
Total	383	

The main grazing system was communal i.e. free range, with main watering points at rivers, and this was practiced by all of the households. The animals are herded by the boys who are employed by the farmers and are paid one female animal for every three years they work (information from farmers). All the animals in the study area were kept in the kraals or animal houses at night. In the rainy season from November to April animals grazed mainly around homesteads and in the dry season from May to October in plains and dambos (open grasslands with few or no trees; their drainage is poor and tend to get water logged in the rainy season). Out of the households that responded to the question on feed supplement, only 31.5% (34/108) of the households keeping cattle, 17.8% (26/146) keeping goats, 41.5% (142/342) keeping chickens and 40.8% (20/49) keeping pigs gave supplements to their animals. These were mainly crop residuals of maize (maize bran and stova) and groundnuts.

Out of the 80 households that had lactating cows only 24 (30%) households milked them. On average they milked 1 litre/cow/day (maximum of 3 litre/cow/day and minimum of 0.2litres/cow/day). Only 1 farmer out of the respondents sold milk.

3.3.12. Important diseases during 1 year prior to the study period (based on clinical diagnosis by farmers).

Out of the 352 disease episodes in cattle encountered during 1 year prior to the study period 38 (10.8%) were identified by farmers as trypanosomiasis and 1 (0.28%) was identified as bloat. Clinical signs were consistent with those recognised for the disease (The Merck Veterinary Manual, 7th Edition, 1991). The rest 313 (88.9%) were not identified by the farmers but only clinical signs were given (Table 3.22).

In chickens 84 cases (4.2%) were identified by names by farmers as Newcastle diseases and 20 (1%) as coccidiosis and 1887 (94.8%) were only described by clinical signs. It is likely that Newcastle disease and coccidiosis were correctly identified because some of the clinical signs matched those in most of the text books (The Merck Veterinary Manual, 7th Edition, 1991). In pigs 50 (49.5%) episodes were identified as African Swine Fever and 51 (50.5%) were not identified but only clinical signs were given by farmers. All the episodes in goats (85) and sheep (2) were not identified by names and only clinical signs were given (Table 3.23).

Table 3.22 Important diseases during 1 year prior to the study (based on clinical diagnosis by farmers)

Livestock	Disease episodes	Clinical signs as seen by farmers	Disease identification by farmers
Cattle	352	Big stomach, standing hair, ear dropping, lacrymation, no appetite, swelling on skin, loss of weight, sweating, hard faeces, limping, swollen eyes, blindness, weak, diarrhoea	- 1 as bloat - 38 as trypanosomiais - 313 not known
Goats	85	No appetite, swellings on the skin, weight loss, standing hair, paralysis, loss of hair, coughing, diarrhoea, swollen legs.	Not known
Sheep	2	Loss of weight, Sudden death	Not known
Pigs	101	No appetite, sudden death, weak tail, paralysis, loss of weight, difficulties in walking, abortion.	- 50 as African Swine Fever - 51 not known
Chickens	1991	No appetite, blindness, coughing, weak, paralysis, watery/whitish/greenish diarrhoea, sudden death, droppings on cloaca, salivation, stretching of the neck, breathing difficulty, swollen face,	- 84 as Newcastle disease. - 20 as coccidiosis - 1887 not known

Table 3.23 Clinical signs of trypanosomiasis

Disease	Clinical signs
Trypanosomiasis	Intermittent fever, lymph-node enlargement, anaemia, weight loss, starring coat, high mortality, abortion, drop in milk production

3.3.13. Livestock deaths during two years prior to the study

In the two years (2003 and 2004) preceding study 94 cattle, 75 goats, 4 sheep, 103 pigs and 2014 chickens died. More chickens 81% (1631/2014) and pigs 93.2% (96/103) died in the dry season and only 10.9% (219) chickens and pigs 6.8% (7)

died in the wet season. On the other hand cattle 73.4% (69/94) and goats 74.7% (56/75) died more in the wet season and in the dry season only 3.2% (3) cattle and 17.3% (13) goats died (Table 3.24).

Table 3.24 Livestock deaths during two years prior to the study

				Season observed		
Livestock	**2003**	**2004**	**Total**	**Dry**	**Wet**	**Both**
Cattle	49	45	94	3	69	22
Goats	21	54	75	13	56	6
Sheep	4	0	4	4	0	0
Pigs	57	46	103	96	7	0
Chickens	1404	611	2014	1631	219	164

3.3.14. Calves born and died during two years prior to the study

In the year 2003 a total of 41 calves were born and 10 died, while in 2004 the number of calves that were born was 46 out of which 6 died. These figures represented calf mortality rates of 24.4% in 2003 and 13.0% in 2004 (Table 3.25).

Table 3.25 Calves born during two years prior to the study.

	Year 2003	**Year 2004**	**Total**
Born	41	46	87
Died	10	6	16
Mortality rate (%)	24.39	13.04	

3.3.15. Drug administration and their source

Most of the farmers, 143 (69.1%) in the study area administered the drugs to animals by themselves (Table 3.26). Only 4 (1.9%) households used the services of Community Livestock Workers (farmers trained to treat other farmers' animals) to administer veterinary drugs. Key informants reported that Veterinary Officers or Livestock Assistants came from other areas to assist once in a while whenever there was a major treatment or vaccination campaign organised by the District or Provincial Office. Sixty (30.0%) households reported using the services of Veterinary Officer and Livestock Assistants to administer drugs for them. These campaigns organised in the area included isometamidium chloride inoculation against trypanosomiasis in cattle, Newcastle disease vaccine in chickens and rabies vaccination in dogs. .

Most of the households (109; 72.2%) obtained their drugs from agro-veterinary shops in the neighbouring districts of Chipata and Katete. The second biggest source was from an Africa Development Bank (ADB) Project, 26 (17.2%) households. Other suppliers were vendors (12 HH; 2.6%) and Community Livestock Workers (4 HH; 2.6%). See Table 3.27. The services provided by the Livestock Assistants and Veterinary Officer under the ADB project were free of charge, where as those of vendors and CLW were paid for (Table 3.27).

Table 3. 26 Veterinary drug administration

People who administer the drugs	Number of Households	Percentage of Households
Farmers	111	53.62
Other farmers	32	15.46
Com. Livestock Worker (CLW)	4	1.93
Livestock Assistants (LA)	44	21.26
Veterinary Officers (VO)	16	7.73
	207	

Com. = Community

Table 3. 27 Veterinary drug source

Source of drugs	Number of Households	Percentage (%) of households
Vendors	12	7.95
Community Livestock Workers	4	2.65
Livestock Assistants/Veterinary Officers (ADB)	26	17.22
Agro-Vet shop	109	72.19
	151	

3.3.16. Drug used during one year prior to the study

The drugs commonly used by the farmers were the trypanocides i.e. diminazene aceturate and isometamidium chloride. Diminazene aceturate was used by 76 (48.7%) households and isometamidium chloride by 32 (20.5%) households. The Newcastle disease vaccine was also reported to have been used in the area by 22 (14.1%) households. Some farmers (8 households) reported using traditional medicines such as salt or a combination of hot chilli and salt to cure chicken diseases. Others used human medicines like septrin (1 household) and a combination of soap and human pain-killer tablets (paracetamol). Only one household reported using de-wormers in cattle (Table 3.28).

Table 3. 28 Common used drugs in the area during one year prior to the study

Name of drug used	Livestock given the drug	Number recovered	Who gave the drug	Number HH used drug
Diminazene aceturate	Cattle (258)	215	Farmer/VA	76
Isometammidium chloride	Cattle (207)	191	Vet/farmer	32
Newcastle disease vaccine	Chickens (193)	86	VO/VA	22
Terramycine	Chikens (20)	20	farmer	1
Hot chilli & salt	Chickens (60)	19	farmer	6
Oxytetracycline	Cattle (17)	13	farmer	8
Salt	Chickens (11)	11	farmer	2
Septrin (human antibiotic)	Chickens (7)	7	farmer	1
Terramycine	Cattle (5)	5	farmer	2
Soap and panado	Chickens (5)	5	farmer	1
Traditional medicine (Chikoswe/Chivungula)	Chickens (45)	2	farmer	2
Diminazene aceturate	Goats (2)	1	farmer	1
Indocide	Chickens (1)	1	farmer	1
Dewormer	Cattle (1)	1	farmer	1
				156

HH =households, VO= Veterinary Officer, VA= Veterinary Assistant

3.3.17. Trypanocidal drug dilution and administration by farmers

Out of 56 households reporting use of isometamidium chloride, 43 (76.8%) households obtained their water to dilute their drugs from a river and 13 (23.2%) households from wells. Fifty-five households (98.2%) who used isometamidium boiled water before diluting the drug and only 1 (1.78%) household did not. Fifty-nine (72%) households who used diminazene aceturate obtained water from rivers, 17 (20.7%) households from wells and 6 (7.3%) from boreholes. Seventy-nine (96.34%) households using diminazene aceturate boiled water and 3 (3.7%) did not before diluting the drug (Table 3.29).

Table 3. 29 Source of water used for diluting the trypanocides.

Isometamidium chloride					Diminazene aceturate				
Water source			Water boiled?		Water source			Water boiled?	
River	Well	borehole	Yes	No	River	Well	borehole	Yes	No
43	13	0	55	1	59	17	6	79	3

For isometamidium chloride, a dose of 0.25 to 0.5mg/kg body weight is recommended for curative treatment and 0.5 to 1mg/kg for preventive treatment (CEVA SANTE ANIMALE manufacturers data sheet, Appendix 5a). Taking average weight of 250kg for an ox, only 3 (5.4%) farmers were giving correct dosage for preventive measure. Forty (71.4%) households gave correct dose for curative treatment but not for prevention. Eight (14.3%) households over-diluted the drug and were under-dosing their animals. Five (8.9%) households added the powder to water until they obtained a colour they thought was the right concentration; this is not recommended (Table 3.30).

Diminazene aceturate is recommended for use at a dose of 3.5mg/kg (1ml/20kg) body weight i.e. by diluting 1.05g of the granules into 12.5 ml of sterile water, and administering 1ml/20kg body weight (Intervet manufacturers data sheet, Appendix 5b). Forty-three (59.7%) farmers administered correct volumes though the dilution was not correct and 21 (29.2%) of the farmers diluted correctly and administered the right dose. Eight farmers (11.1%) over diluted the drug and under-dosed the animals (Table 3.30).

Table 3. 30 Trypanocidal drug dilution and administration by farmers

Isometamidium chloride			Diminazene aceturate		
Dilution g/ml	**Dosage ml/adult ox**	**Number of HH**	**Dilution g/ml**	**Dosage ml/adult ox**	**Number of HH**
1/50+	5-15+	3	1.05/10	10+	43
1/70	7	1	1.05/12.5+	12.5 +	9
1/80	8-10	8	1.05/12.5+	12+	9
1/96+	12+	1	1.05/12.5+	15+	2
1/100+	10+	28	1.05/14.5+	15+	1
1/100+	12+	2	1.05/15	10	2
1/120	15	4	1.05/20	20	3
1/125	12.5	1	1.05/5	10	1
1/150	20	1	1.05/8	10	1
1/160	20	2	1.05/8	8	1
Added to colour*	10-50	5	No response		29
No response		32			
Total		**88**	**Total**		**101**

+ right dilution and dosage within datasheet recommendations
*** used a teaspoon or groundnut shell to add powder to water till good colour is obtained**

3.3.18. Farmers perception of tsetse and tick problem

Most of the respondents 76.9% (385) perceived tsetse flies were a problem in the area while 61.5% (308) said ticks were a problem. Only 10.4% (52) farmers use dip or spray chemicals to control ticks. The most commonly reported method of tick control was hand picking ticks from the animals (Table 3.31)

Table 3. 31 Farmers perception of tsetse and tick problem. HH = households

	Yes	No
Perceived problem		
Tsetse	385 (76.9%)	116 (23.2%)
Ticks	308 (61.5%)	193 (38.5%)
Tick control method		
Hand picking	117 (23.4%)	384 (76.5%)
Dip or spray with acaricide	52 (10.4%)	449 (86.6%)

Figures in parentheses are percentages of farmers responding to this question

3.4. Discussion

Household demographic characteristics and livestock herd structure were studied in the study area in Mambwe district, Eastern Province, Zambia. There were 980 households in the area and of those 501 households were interviewed. There were 685 cattle, 1350 goats, 46 sheep, 262 pigs and 7661 chickens in the study area

3.4.1. Households demographic characteristic

In the study area there were more male-head (80.9%) households than female-head (19.1%) households despite the (2,103) population of woman very similar to that of men (2,034). This is expected because the people who are settling in this study area are mostly from the Ngoni tribe who practice a patrilineal system of inheritance in Zambia. Patrilineal system of inheritance is where inheritance is by the eldest son when the man is married to one wife and if he is married to more than two wives it will be the eldest son from the first wife (Nsemimwe, 2006). There were more households with small family sizes (484; 49.3%) compared with those that had medium (325; 33.2%) family size or large (171; 17.4%) family size. The average household size was 8 persons/household.

Approximately half the households were headed by the people who had migrated into the area, and of those, most had done so in the last 5 years. They migrated into the area in search of fertile land and grazing areas for their animals. The semi-plateau is characterized by sand-loam soils while the valley contains alluvial soils that are quite fertile but prone to erosion (DACO, Mambwe district report, 2004). On the plateau where immigrants are coming from, land is becoming scarce as cattle population is increasing. This may in part be the result of tsetse control activities that have been implemented on the plateau, much of which were undertaken by The Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) that operated in the area from the late 1980s and 1990s (RTTCP, 1995) and some by the Assistance of Veterinary Services to Zambia (ASVEZA). Cattle population density on the plateau in 1997 was 5 head of cattle/km^2 (Van den Bossche and Staak, 1997) and in 2004 the cattle density was 10 cattle/km^2 (Van den Bossche *et al*, 2004).

The main source of income for the people in this area was mixed crop-livestock farming (84.0%) mainly from cotton growing. The average income per household per annum was about K1,849,131 (US$ 377.85) or approximately US$1.03 per day. Most of the people living in this area are poor and their houses (66.7%) were of poor quality and made of mud walls and grass thatched roof. The average land holding size per household in the area was 5.86acres of which 74.1% was allocated for crop production and only 9.9% for animal grazing. In the study area most of the households were situated mainly along one main road that stretched from the base of the valley all the way to the plateau (Figures 3.1a,b). This gravel road has no public transport and farmers rely on other farmers' vehicles when ever they want to go to other areas.

3.4.2. Crops grown, fertilizer and manure usage

The land in the valley has maintained its natural fertility because few households use artificial fertilizer. The main crops grown in the area were maize, cotton, groundnuts, rice, sunflower, sorghum, soya beans, tobacco, cassava, cowpeas and beans. The majority of the farmers growing these crops do not use livestock manure and not artificial fertilizers. This study revealed that only 6 (1.2%) households used artificial fertilizer and 53 (10.6%) households used livestock manure. The Chiefs in the area do not encourage the use of artificial fertilizers (personnel communication, District Agriculture Coordinator, Mambwe district).

Cotton is widely grown in the area because of the availability of the seed and pesticides that are given to the farmers on loan by the two major companies (Dunarvant and Clark cotton company) operating in Eastern Province of Zambia.

The abundance of wildlife in the area is evident from the number of households 113 (22.55%) that reported moving from their permanent homes to temporary shelters close to their crop fields during the rainy season. Animals do eat and destroy crops as they feed hence the need for these farmers to move temporary and stay near their fields to make it easier for them to constantly chase away these wild animals. The

wild animals play a very significant role in the epidemiology of trypanosomiasis in this area as they act as reservoir host of the trypanosomes (Heisch, *et al*, 1958).

3.4.3. Mosquito control and number of cases

About 73.2% of the farmers in the area used mosquito bed nets and 79.4% use anti-malarial drugs to control malaria. The mean number of malaria cases was 4/cases/household/year. Most of the households walked very long distances (on average 10Km) to medical centres for medical treatment and sick patients were mainly transported by either ox-carts or bicycles (personal observation). Spraying mosquitoes with synthetic pyrethroids as a method to control malaria was not practiced among the respondents in the area but the use of deltamethrin to control cotton pests could have some effect. It has been demonstrated that using mosquito bed nets impregnated with deltamethrin has great effect in reducing the number of malaria cases (Joshi *et al*, 2003).

3.4.4. Livestock ownership, management and veterinary drug use.

3.4.4.1. Livestock ownership

On average there was 1 ox per household and only 117 (11.9%) households kept cattle at the time livestock census was conducted prior to the study. A communal free grazing system was practiced by all the farmers and animals were mainly watered in rivers. Most cattle in the area have been recently introduced by immigrants, and on average farmers have been keeping them for just 5 years. Farmers kept more oxen 339 (49.5%) than any other category probably as an indication of the farmers' desire to increase the numbers of draught animals and hence ploughed crop acreage. Only 24 (30%) households out of the 80 households that had lactating cows milked them and on average milked 1litre/cow/day. This amount is typical of tsetse infested area (Gideon Kasalagila, unpublished report, Machila *et, al*, 2003; Thuranira, 2005). Only 1 farmer out of the respondents sold milk, a reflection of the small volume of milk produced, a situation observed in most of the areas that are tsetse infested (Kristjanson *et al*, 1999).

Chickens were the most commonly kept livestock (35.8%) followed by goats (15.1%) and very few households kept sheep (0.82%). Pigs were kept by 8.4% of the households. Lack of money (49.4% of households) and secondly tsetse flies (15.7% of households) were hindering most of the farmers from owning livestock in the area. Similar observations were made by Doran (2000). At night all the animals were kept in animal houses.

3.4.4.2. Perception of fly and tick problems

Most of the farmers in the area perceived tsetse (76.8%) and ticks (61.5%) to be a problem. Farmers are living among the tsetse flies and have come to know them. This is not surprising because this area is located in the Game Management Area (GMA). Most of it is in the Luangwa valley where tsetse and trypanposomiases cases have been reported. The first documented human case of sleeping sickness that was recorded in 1909 was from this valley (Buyst, 1977).

3.4.4.3. Animal diseases in the area (report based clinical diagnosis by farmers)

For most species of livestock, farmers could name only the most commonly reported diseases, such as Newcastle disease in poultry, or bovine trypanosomiasis. For other diseases, clinical signs were described. In cattle the most commonly identified disease by the farmers was trypanosomiasis (38/352 disease episodes; 10.79%). Some diseases were not identified by the farmers but only clinical signs were given. The major clinical signs seen in ruminants as reported by these farmers included staring coat, lacrymation, loss of weight, sweating, hard faeces, limping, swollen eyes, blindness, weakness, abortion, sudden death and diarrhoea. Many of these clinical signs, e.g. starring coat, loss of weight, abortion, are consistent with trypanosomaisis, although some of the clinical signs such as hard faeces, limping,

swollen eyes, blindness, sudden death and diarrhoea would more likely be caused by other diseases (The Merck Manual, Seventh Edition, 1991)

Most of the diseases (50/101; 49.5%) in pigs were identified as African Swine Fever and others were only described by clinical signs which included no appetite, sudden death, weak tail, paralysis, loss of weight, difficulties in walking and abortion. In chickens 4.2% (84/1991) were identified by farmers as Newcastle Disease and common clinical signs cited included loss of appetite, blindness, coughing, weak, paralysis, watery/whitish/greenish diarrhoea, sudden death, droppings on cloaca, salivation, stretching of the neck, breathing difficulty, and swollen face. These clinical signs are consistent with Newcastle disease. In the area chicken (1631/2018 ; 81%) and pigs (96/103; 93.2%) were dying more in the dry season while cattle (69/94; 73.4%) and goats (56/75; 74.7%) died more in the wet season. The mortality rate in calves in the area during two years prior to the study was of 24.4% in 2003 and 13.0% in 2004.

3.4.4.4. Common drugs used by the farmers in the area

The common drugs used by the farmers (109/156; 69.9% of the households) in the area were the trypanocides (isometamidium chloride and diminazene aceturate). This is not surprising because most of them (76.8%) perceived tsetse (trypanosomiasis) to be a problem. Furthermore most of the clinical signs they reported corresponded to those of trypanosomiasis (Tables 3.22 and 3.23), and hence this choice of treatment was probably appropriate. The other reason is that in Eastern Province isometamidium and diminazene are known as 'wonder drugs' by farmers and are not only used to treat trypanosomiasis, but also any other diseases (Van den Bossche et al., 2000).

Farmers' knowledge in drug dilution and administration was limited because most of them were not using the correct amount of water to dilute the trypanocide. Only about 50% of the households gave the animals the correct dose of diminazene aceturate. For isometamidium chloride only 5% gave the correct dose for prevention

and 71.43% of the households gave correct dose for curative treatment but not for prevention (Table3.30).

Hand-picking of ticks from the animals was the most common used method for tick control practiced by the farmers. About 23.4% of the households reported to hand-pick ticks from the animals and only 10.4% households used dip or spray chemicals to control ticks.

3.4.4.5. Veterinary services

The area lacked good veterinary services and at the time this study was conducted there were no veterinary doctors or veterinary assistants. This resulted in the farmers (69.1%) relying on themselves to treat their animals most of the times. Some farmers had very little knowledge of dilution and administration of the most commonly used drugs (trypanocides) in the area as evident from the finding of this work, a situation that may lead to drug resistance if not quickly checked (Geerts and Holmes, 1998). It is likely that very big oxen were been under-dosed and younger ones over-dosed as administration of the drug was not based on the animals' weight using either weighing scales or bands. Farmer training in weight estimation, drug dilution and administration is highly recommended in this area if animals have to be treated properly.

Most of the farmers (72.2%) bought their drugs from agro-vet shops from outside the district and paid for them with cash. There were no shops within the study area where veterinary drugs were sold. On one occasion in 2004/2005 the African Development Bank (ADB) project under the Department of Veterinary and Livestock Development provided trypanocides to cattle, Newcastle vaccine to chickens and Rabies vaccines to dogs at no cost to the farmers. It is important that drugs are made available to the farmers at the nearest shops to them since the number of animals they are keeping is increasing. Lack of transport in the area makes it difficulty for the farmers to get to the shops outside the district.

3.4.4.6. Cotton growing; effect on trypanosomiasis and malaria

The growing of cotton could have some significant effect in reducing the number of tsetse flies and the mosquitoes in the area. This is because the pesticides the farmers are using to control cotton pests are cypermethrin and deltamethrin. These chemicals have been successfully used to control mosquitoes (Joshi *et al*, 2003) and tsetse (Chizyuka and Luguru, 1986) the vectors that carry plasmodium and trypanosomes parasites that cause malaria and trypanosomiasis respectively.

4. CHAPTER FOUR

4. FACTORS AFFECTING THE EPIDEMIOLOGY OF ANIMAL TRYPANOSOMIASIS IN MAMBWE DISTRICT, EASTERN PROVINCE, ZAMBIA

4.1. Introduction

Tsetse- transmitted animal trypanosomiasis is a debilitating and commonly fatal disease of domestic livestock. Understanding how the disease is spread and maintained in a population is important because effective disease control and management depends heavily upon knowledge of the epidemiology of the disease (Van den Bossche, 2001, Hutchinson *et al.*, 2003). Several trypanosomiasis surveys have been conducted on the plateau in Eastern Province of Zambia to determine the prevalence of trypanosomiasis in livestock and many cases of the disease have been reported (Sinyangwe *et al*, 2004; Machila *et al.*, 2001; Mubanga, 1996). In most of the trypanosomiasis surveys that have been conducted where the disease occurs in Zambia, people have had no interest in analysing the prevalence of trypanosomiasis in livestock that are kept in different combinations. These could be either a combination of cattle and small ruminants or pigs and small ruminants or combination of cattle and pigs or all three types of these domestic animals. In Zambia, in most cases, animal trypanosomiasis surveys have been carried out in cattle only. Small ruminants and pigs have been left out of the surveys. This is because farmers and most of the veterinary officials pay less attention to smaller animals (personal observation), but what impact do these ignored animals have on the epidemiology of trypanosomiasis? The effect they have on the spread and maintenance of trypanosomiasis in animal population can only become apparent if only they are included in the epidemiological studies. Such information can be obtained by studying the prevalence of the disease in domestic animals kept under different combination of species.

For the purpose of this study, a cross-sectional survey was conducted to provide information on the disease prevalence from the valley to the plateau in Mambwe District, Eastern Province of Zambia. The survey provided information on the species of trypanosomes circulating in the livestock in the area. Haemoglobin (Hb) values of each and every individual animal were also obtained from this survey. This study aims at adding further information to the pool of knowledge that already exists on the epidemiology of domestic animal trypanosomiasis in Zambia and in other places having similar conditions. The results reported here are based on the

trypanosomiasis prevalence that were obtained in 649 cattle, 811 goats, 58 sheep and 177 pigs that were sampled during the survey that was conducted in Eastern province of Zambia. The survey was carried out in the four species of animals at the same time in the year 2005.

4.2. Objective of the study of this chapter

The overall objective of the work presented in this chapter was to study the epidemiology of animal trypanosomiasis from the valley to the plateau in Eastern province, Zambia.

The specific objectives were as follows:

1. To determine the species of trypanosomes found in the livestock in the area and distribution patterns from the valley to the plateau.
2. To determine the relationship between trypanosomiasis prevalence and livestock farming practice (combination of species of livestock kept), altitude, household size and trypanocidal drug treatment.

4.3. Material and methods

4.3.1. Study area

The study area was situated between 31°04'- 32°05' E and 13°05' – 13°50' S in Mambwe District, Eastern Province of Zambia. This area is situated in the Luangwa valley and stretches all the way from the base of the valley to the plateau. Most of the villages are concentrated along one main road that cuts through the valley in a transect manner (Chapter 3, Fig. 3.1a and 3.1b). The area is infested with three species of tsetse flies namely *Glossina morsitans morsitans*, *Glosssina pallidipes* and *Glosssina brevipalpis*. Cattle have recently been introduced into the area (1st quarterly report, 2005, DACO, Mambwe) and rely heavily on trypanocidal drugs for their survival. Other livestock kept are rarely or not treated at all due to the fact that many of these farmers are poor and can not afford the cost of treatment. No vector

control activities have been deployed in this area. See Chapter 2, Section 2.2.3 and Chapter 3, Section 3.2.1.

4.3.2. Selection of the animals and analysis of the trypanosomiasis prevalence in animals belonging to households keeping different combination of livestock species.

Selection of the animals to be sampled was based on the results of the census that was conducted in the area prior to sampling of the livestock (Refer to chapter 3). All sheep, pigs and cattle were sampled as there were relatively few in the area. Only a randomly selected proportion of goats were to be sampled. Selection of goats was based on the following; all goats in the households with pigs were to be sampled and only a randomly selected sample from households with small ruminants only (category 3) and a combination of cattle and small ruminants (Category 5). See Table 4.1. The minimum number of goats that was required from each of these two categories to provide 95% confidence of detecting at least one positive at prevalence of 5% was calculated to be 293 (Cannon and Roe, 1982; Thrusfield, 2000). Previous trypanosomiais survey conducted in the area at Kakumbi indicated incidence of 18% and 7% (Bealby, *et al*, 1996).

The minimum number of the sample size was determined by the following equation;

$$\mathbf{n = \frac{1.96^2 P_{exp}(1- P_{exp})}{d^2}}$$

n	= required sample size
p_{exp}	= expected prevalence of 12.5%.
d	= desired absolute precision (5%)
1.96	= standard errors associated with confidence intervals.

$$n = \frac{1.96^2 \times 0.12 (1- 0.12)}{0.05^2} = \mathbf{162}$$

Step 1 **Adjust for the estimated Sample Design Effect** (Aday, 1996)

The figure was adjusted by multiplying by 1.3

n = 162 x 1.3 = **211**

Step 2 **Adjust for the Expected Response Rate.**

It was then adjusted for expected response rate by dividing by 80%.

211 ÷ 0.80 = **264**

Step 3 **Adjust for the Expected Proportion of Eligibles**

This was done by dividing by 90%.

264 ÷ .90 = **293**

Efficient sample size calculations are based on an estimate of the sample size required to limit sampling variability to the desired level. Efficient sample size calculations assume simple random samples. Therefore, sample designs other than simple random sampling have an impact, called design effects, on sampling variability. As a result of this impact, design effects are important considerations when determining sample size. The design effect represents the cumulative effect of design components such as stratification, unequal weighting, and clustering, and will differ for each design. For example, sampling variability increases when cluster sampling is used rather than simple random sampling. The design effect is a direct way of addressing the impact of design on sampling variability. The design effect can be multiplied by the expected sampling variance in the calculation of an efficient sample size to adjust for the impact of the design.

The disease prevalence in livestock was analysed based on households keeping different combination of livestock species (Table 4.1). Other parameters that were recorded for each individual animal sampled included haemoglobin values (Hb), species and breed of animal, sex, date they were last treated with trypanocides, condition of the coat and lymph node size.

The combination of livestock kept by the farmers was as follows:

1. Cattle only
2. Small ruminants only (i.e. goats or sheep)
3. Pigs only
4. Combination of cattle and small ruminants
5. Combination of cattle and pigs
6. Combination of small ruminants and pigs
7. Combination of cattle, small ruminants and pigs

Table 4. 1 Livestock population in the study area, Mambwe District as per census conducted in May 2005.

Category	Livestock combination	Cattle	Goats	Sheep	Pigs	Number of HH
	None (no livestock)	0	0	0	0	**708**
1	**Cattle only**	365	0	0	0	**66**
2	**Small ruminants only**	0	705	8	0	**104**
3	**Pigs only**	0	0	0	118	**25**
4	**Cattle & small rum.**	235	394	38	0	**41**
5	**Cattle & Pigs**	37	0	0	34	**8**
6	**Small rum & pigs**	0	128	0	67	**16**
7	**Cattle, Small rum & pigs**	48	123	0	43	**12**
		685	**1350**	**46**	**262**	**980**

4.3.3. Sampling of animals in the field

Since the survey included small animals i.e. pigs, goats and sheep, the only easiest way we could access them was by visiting the farmers household to household. Farmers were given the dates when the animals were to be sampled well in advance. This was done to allow the farmers to gather their animals together and have them enclosed in their kraals ready to be sampled. Notice was given to the farmers about two to three days before the sampling date. Finding the location of the households for

the farmers was not difficult. The positions (latitudes and longitudes) of the households were geo-referenced using a Geographical Positioning System (GPS) during the animal and human census exercise that was conducted in the area just before the survey. All the species of animals were sampled on the same day where a farmer was keeping more than one species of animals.

Blood was collected from them for the preparation of thick and thin blood smears in the field and blood spots. The blood was collected from the peripheral ear vein using heparinised capillary tubes after the vein was punctured by a sterile lancets. Detailed of sampling technique is described in chapter two of this thesis.

4.3.4. Detection of trypanosome infection by parasitological and molecular methods

4.3.4.1. Parasitological method

The thick and thin blood smears were made on the same slide on site in the field, dried away from direct sunlight and packed in the slide boxes for fixing and staining at the laboratory. In the laboratory the thin smear were fixed with methanol for 3 minutes and thick smears haemolysed by dipping in water for about 2 minutes. They were then dried and stained with 10% giemsa for 30 minutes. After staining the slides were dried and examined for trypanosomes using a 10x100 magnification (Paris *et al.*, 1982).

4.3.4.2. Molecular method

Blood was collected from the peripheral ear vain into the capillary tubes and used to make blood spot on the Whatman FTA® cards. Whole blood was collected from the jugular vain into the vacutainer tubes and later on blood spot made using capillary tubes onto filter papers. When making the blood spots the team made sure that this was done in a shade. After making the spots they were packed in special storage bags with desiccant and stored for future use in a cool place. PCR assays (Cox *et al.*, 2005) was done at the University of Edinburgh.

4.3.4.3. Haemoglobin (Hb) readings

Blood from a peripheral of the ear-vain was collected into the HemoCue Hb 201 Microcuvette and placed into the slot of the HemoCue Hb analyser. The haemoglobin value was then read directly from the Hemo Cue Hb 201 Microcuvette. The values were read in g/dl (see chapter 2).

4.3.4.4. Effect of cotton growing on trypanosomiasis prevalence in cattle

It is hypothesized that the closer the household is to the cotton field the lesser the prevalence of trypanosomiasis. The effect of cotton growing on trypanosomiasis prevalence was observed by asking the farmers how long it take them to walk from their homes to their cotton fields. Time to walk to the field was used as a measure since few farmers in the area are not able to estimate distance in kilometres. Cotton is sprayed with deltamethrin a chemical that also kills tsetse flies the vector of trypanosomes.

4.3.5. Data handling and statistical analysis

Data on trypanosome infections was compiled, entered and stored in Excel spreadsheet for analysis. Graphs were prepared using Microsoft Excel worksheets. Statistical comparisons by proportion was done by Chi-square tests using Minitab 13.2 software. Where the sample size was small and expected values were less than 5, comparisons of the proportion was done by Fisher's exact test using R. version 2.4.1 software package. The comparison of mean haemoglobin concentration values was done using Two-sample t-test with Minitab 13.2 software.

4.4. Results

4.4.1. Livestock sampled

A total of 649 cattle, 811 goats, 58 sheep and 177 pigs were sampled in the area. Livestock species combination and the number of households keeping these animals were as shown in Table 4.2 below.

Table 4. 2 Livestock sampled and households owning the animals.

Livestock combination	Cattle	Goats	Sheep	Pigs	Number of Households
Cattle only	326	0	0	0	**60**
Small ruminants only	0	412	11	0	**93**
Pigs only	0	0	0	79	**25**
Cattle & small rum.	245	288	47	0	**38**
Cattle & Pigs	13	0	0	15	**8**
Small rum & pigs	0	63	0	23	**14**
Cattle, Small rum & pigs	65	48	0	60	**12**
	649	**811**	**58**	**177**	**250**

4.4.2. Prevalence of trypanosomiasis in livestock species.

Animals were considered to be positive when found positive by either microscopy examination or PCR analysis or both methods.

4.4.2.1. Overall trypanosomiasis prevalence in livestock in the area regardless of the combination of animal species kept.

The overall trypanosomes infection in the area regardless of the combination of animal species kept was more in cattle 216/649 (33.3%, 95%CI: 29.8% to 37.0%) followed by sheep 16/58 (27.6%, 95%CI: 17.8% to 40.2%). The prevalence in goats was the lowest at 83/811(10.2%, 95%CI: 8.30% to 12.5%). In pigs it was 37/177 (20.9%, 95%CI: 15.6 to 27.5%) (Figure 4.1F). The order in this case was Cattle >

Sheep > Pigs > Goats. The prevalence in the animal species differed significantly ($P < 0.05$, $\chi^2 = 118.097$ with 3 d.f.).

4.4.2.2. Trypanosomiasis infection in livestock in households keeping individual types of animals

The prevalence of trypanosomiasis in livestock in households that kept individual a single type showed that cattle had the highest prevalence 91/326 (27.9%, 95%CI; 23.3% to 33.0%) followed by pigs at 17/79(21.5%, 95%CI: 13.9% to 31.8%). Goats had the lowest prevalence 38/412(9.22%, 95%CI: 6.8% to 12.4%). The prevalence in sheep was 2/11(18.2%, 95%CI: 5.1% to 47.7%). The order of prevalence was as follows; Cattle > Pigs > Sheep > Goats (Figure 4.1A). The prevalence in the animal species differed significantly ($P < 0.05$, $\chi^2 = 45.5$ with 3 d.f.).

4.4.2.3. Trypanosomiasis prevalence in livestock in households keeping a combination of cattle and small ruminants

In households where farmers were keeping a combination of cattle and small ruminants the prevalence was higher in cattle 104/250 (41.6%, 95%CI: 35.7% to 47.8%) while that of small ruminant was 43/288 (14.9%, 95%CI: 11.3% to 19.5%) in goats and 14/47 (29.8%, 95%CI: 18.7% to 44.0%) in sheep. The order again was Cattle > Sheep > Goats (Figure 4.1B). The prevalence in cattle and small ruminant was significantly different ($P < 0.05$, $\chi^2 = 47.851$ with 2 d.f.).

4.4.2.4. Trypanosomiasis prevalence in livestock in households keeping a combination of cattle and pigs

In households where the farmers kept a combination of cattle and pigs, the prevalence was high in pigs 4/15 (26.7%, 95%CI: 10.9% to 52%) and in cattle it was 3/13 (23.1%, 95%CI: 8.2% to 50.3%). The order was Pigs > Cattle (Figure 4.1C). Although the proportion of pigs that were infected was high than the cattle infected this was not statistically significant ($P > 0.05$, $\chi^2 = 0$ with 1 d.f.).

4.4.2.5. Trypanosomiasis prevalence in livestock in households that were keeping a combination of goats and pigs.

Where a combination of small ruminants and pigs were kept, the prevalence was high in pigs 5/23 (21.7%, 95%CI: 9.7% to 41.9%) while that of the goats was 1/63 (1.59%, 95%CI: 0.3% to 8.5%). The order was Pigs > Goats (Figure 4.1D). The prevalence was significantly different ($P < 0.05$, $\chi^2 = 7.67$ with 1 d.f.).

4.4.2.6. Trypanosomiasis prevalence in livestock in households keeping a combination of cattle, small ruminants and pigs

In households that were keeping all the species of livestock the prevalence was high in cattle 18/65 (27.7%, 95%CI: 18.3% to 39.6%) followed by pigs at 11/60 (18.3%, 95%CI: 10.6% to 29.6%). Small ruminants (goats) were the lowest with prevalence of 1/48(2.08%, 95%CI: 4% to 10.9%). The order followed was Cattle > Pigs > Goats (Figure 4.1E). The prevalence in the animal species differed significantly ($P < 0.05$, $\chi^2 = 12.696$ with 2 d.f.).

4.4.2.7. The effect on trypanosomiasis prevalence of keeping cattle with other species of livestock.

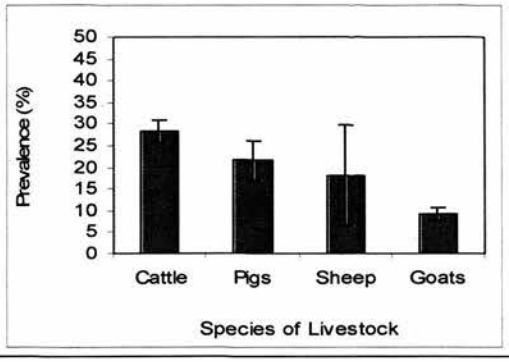
Cattle that were kept in households that had goats had higher prevalence 104/250 (41.6%) compared with those that were kept on their own 91/321 (28.4%), those that were kept with pigs 3/13 (23.1%) or with both small ruminants and pigs 18/65 (27.7%). This was highly significant ($p= 0.0052$, $\chi^2 = 12.832$, 3 d.f.). The difference in prevalences of trypanosomiasis among cattle kept on their own (28.4%), with pigs (23.1%), with both small ruminants and pigs (27.7%) was not statistically different ($p=0.915$, $\chi^2= 178$, 2 d.f.), (Table 4.3 and Figure 4.1).

Table 4. 3 Prevalence of trypanosomiasis in different livestock species combination.

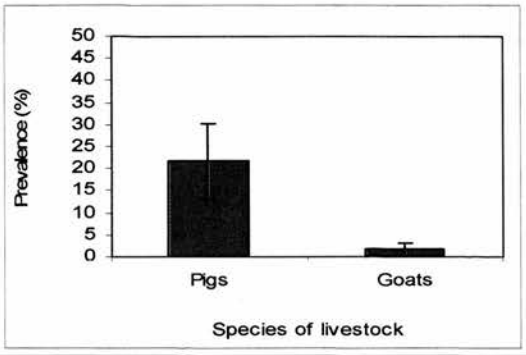
livestock combination	Cattle			Goats			Sheep			Pigs		
	Number sampled	Number positive	Prev. (%)	Number sampled	Number positive	Prev. (%)	Number sampled	Number positive	Prev. (%)	Number sampled	Number positive	Prev. (%)
Individual species	321	91	**28.4**	412	38	**9.22**	11	2	**18.2**	79	17	**21.5**
Cattle and small ruminants.	250	104	**41.6**	288	43	**14.9**	47	14	**29.8**	-	-	-
Cattle and pigs	13	3	**23.1**	-	-	-	-	-	-	15	4	**26.7**
Small ruminants and pigs	-	-	-	63	1	**1.59**	0	0	-	23	5	**21.7**
Cattle, small ruminants and pigs	65	18	**27.7**	48	1	**2.08**	0	0	-	60	11	**18.3**
Total	649	216	**33.28**	811	83	**10.23**	58	16	**27.58**	177	37	**20.9**

Figure 4. 1 Prevalence of trypanosomiasis in different livestock species combination.

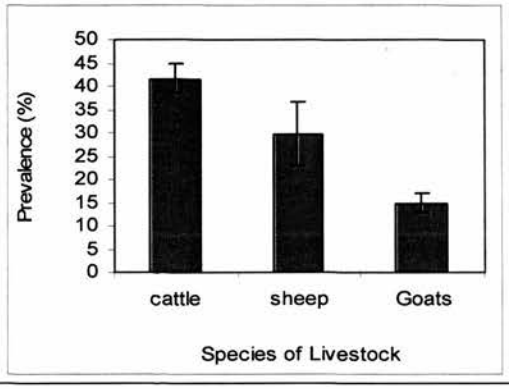
A. Trypanosomiasis prevalence by households keeping individual animal species



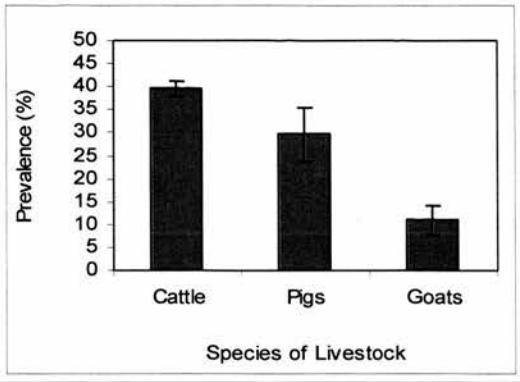
D. Trypanosomiasis prevalence by households keeping a combination of Small ruminants and pigs



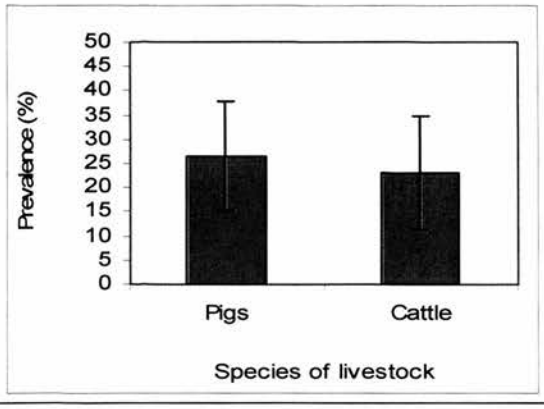
B. Trypanosomiasis prevalence in livestock in households keeping a combination of cattle and small ruminants



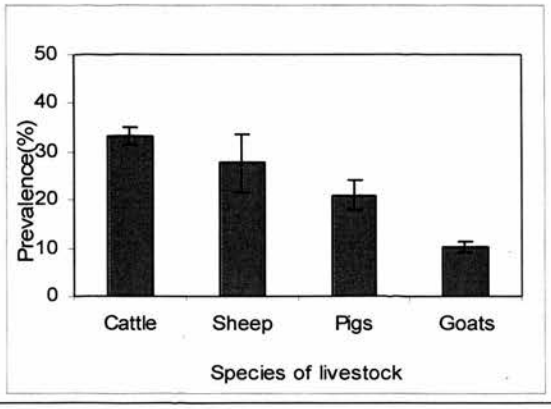
E. Trypanosomiasis prevalence in livestock in households keeping a combination of cattle, small ruminants and pigs



C. Trypanosomiasis prevalence in livestock in households that were keeping a combination of cattle and pigs.



F. Overall trypanosomiasis prevalence in the area regardless of the combination of animals species kept



4.4.2.8. Agreement of PCR with thick and thin smears results in cattle.

Using one punch from the blood spot on the filter paper for the PCR run, the kappa value (concordance between PCR and thick and thin smear) was 0.44. The whole thin and thick smears on each slide were screened for the parasites. Kappa value assesses the concordance between different tests. Kappa ranges from 1 (complete agreement) to 0 (agreement due to chance). In this case the kappa value of 0.442 was moderate. PCR technique detected more positive than the thick and thin smears despite using one punch of the blood spot. Thick and thin smear examination revealed a prevalence of 18.0% (117/649) in cattle while PCR revealed a prevalence of 28.4% (184/649).

Table 4. 4 Agreement between PCR and thick and thin smear (cattle results).

	PCR Examination Results			
		Positive	**Negative**	**Total**
Thick and thin smear results	**Positive**	85	32	117
	Negative	99	433	532
		184	465	649

Kappa value = 0.44

4.4.3. Trypanosomiasis prevalence in livestock by age group

4.4.3.1. Trypanosomiasis prevalence in cattle

Prevalence in cattle of all ages and sexes was broadly similar except for cattle 1-12 months where the infection rate was higher in males and lower in females (Table 4.5, Figure 4.2). Although the proportion of cattle that was infected were less in young animals than the older ones, at 0.05% level of significance, there was no linear trend between the proportion of the herds affected and the age group. ($P = 0.136$, $\chi^2 = 0.136$, 1 d.f)

When the prevalence was compared in different sexes of cattle, the difference was only observed in the age group 1-12 months. The difference was statistically significant (*P*-value = 0.046, χ^2 = 3.982 with 1 d.f.). In the older age groups, 13-24 months and >24 months, there was no difference in the prevalence of trypanosomiasis between the male and female groups (P>0.05, p-value is 0.97). See Table 4.5 below and Figure 4.2.

Table 4. 5. Trypanosomiasis prevalence (%) in cattle by age group

	1-12 months			13-24 months			>24 months		
Sex	Number sampled	Nº +ve	Prev. (%)	Number sampled	Nº +ve	Prev. (%)	Number sampled	Nº +ve	Prev. (%)
Male	26	11	42.3	28	10	35.7	393	130	33.1
Female	20	3	15.0	10	3	30.0	172	59	34.3
Total	46	14	30.4	38	13	34.2	565	189	33.4

Prev. = prevalence, Nº +ve = Number of animals positive

4.4.3.2. Trypanosomiasis prevalence in goats, sheep and pigs

In Goats, the infection rate increased with age and at the 0.05% level of significance, there was a linear trend between the proportion of affected animals and the age of the goats (*P*-value = 0.0238, χ^2 = 5.01 with 1 df). Although there were fewer infections in young animals than the older one in both the sheep and pigs, at the 0.05% level of significance, there was no linear trend between the proportion of affected animals and age, in sheep (*P*-value = 1.8178, χ^2 = 0.1776 with 1 df) and pigs (P value = 0.2328, χ^2 = 1.4238 with 1 df), (Table 4.6 and Figure 4.3).

Table 4. 6Trypanosomiasis prevalence (%) in goats, sheep and pigs by age group

	1-6 months			7-12 months			>12 months		
	Number sampled	Nº +ve	Prev. (%)	Number sampled	Nº +ve	Prev. (%)	Number sampled	Nº +ve	Prev. (%)
Goats	182	12	6.59	192	17	8.8	437	54	12.4
Sheep	13	2	15.4	6	1	16.7	39	13	33.3
Pigs	79	12	15.2	74	20	27.0	24	5	20.8

Prev. = prevalence, Nº +ve = Number of animals positive

Figure 4. 2 Trypanosomiasis prevalence in cattle by sex and age group

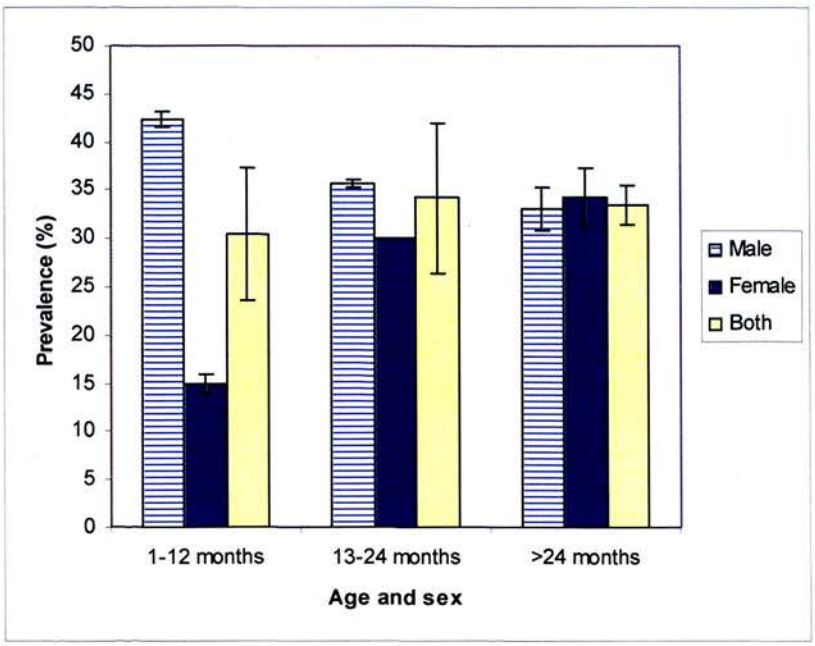
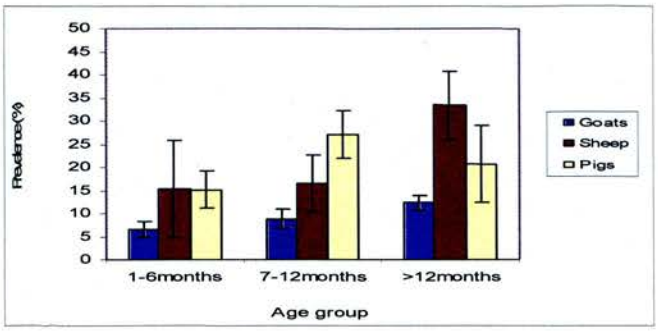


Figure 4. 3 Trypanosomiasis prevalence in goats, sheep and pigs by age group



4.4.4. Trypanosomiasis prevalence in livestock at different altitudes

As you move from the base of the valley to the plateau the temperature increases and also there is a change in the vegetation type. The average temperature on the plateau is (Mean maximum 25°C and mean minimum 5°C) and at the base of valley is (Mean maximum 35°C and mean minimum 13°C). The mean monthly rainfall on the plateau is 700mm and in the valley is 100mm. The vegetation on the plateau is predominantly 'Miombo' woodland (*Brachystegia*, *Julbernardia* spp) and 'Munga' woodland (*Acacia*, *Combretum*, *Terminalia* species) on the plateau and gradually gives way to 'Mopani' woodland (*Colophospermum mopane*) in the valley. On the plateau tsetse habitat has been modified due to intensive human land-use (Van den Bossche, 2001).

Habitant modification by intensification of land use by human is of huge importance in the epidemiology of trypanosomiasis (Jordan, 1986). Habitant modification results in gradual decrease in density of tsetse and wild hosts in settled areas (plateau) which become unsuitable for the fly. These results in the increase in number of cattle and cattle become important in tsetse diet (Van den Bossche, 2001). Such scenario is presented as you approach the plateau where the study was conducted. This situation will present itself in the valley in the near future as farmers are continuously moving into the area (Chapter 3).

In cattle the prevalence was 44.2% at altitudes 501-600 meters above sea level and 30.7% at altitudes of 601-700m and between altitudes 701-800m it was 26.3%. At 0.5% level of significance we can say there is a linear trend between the affected herds and the increase in altitude (χ^2 =31.188, d.f. = 1, *p*-value = 0.0006).

The prevalence of trypanosomiasis in the other species of animals sampled i.e. goats and pigs followed the same pattern from the valley to the plateau (Table 4.7, and Figure 4.4). In goats the prevalence was 11.11% at altitudes 501-600m and in the altitude 601-700m the prevalence was 10.93% and was 3.57% in the altitudes 701- 800m. Since the prevalence at altitudes 501-600m and 601-700m were almost the same and not statistically different at 0.05% (χ^2 =0.006, d.f. = 1, *p*-value=0.940), the results of these two groups were pooled together and compared with the results in the altitude 701-800m. After doing so, at 0.05% level of significance there was a linear trend between the affected goats and the increase in altitude (χ^2 = 441, d.f. = 1, p-value < 0.001).

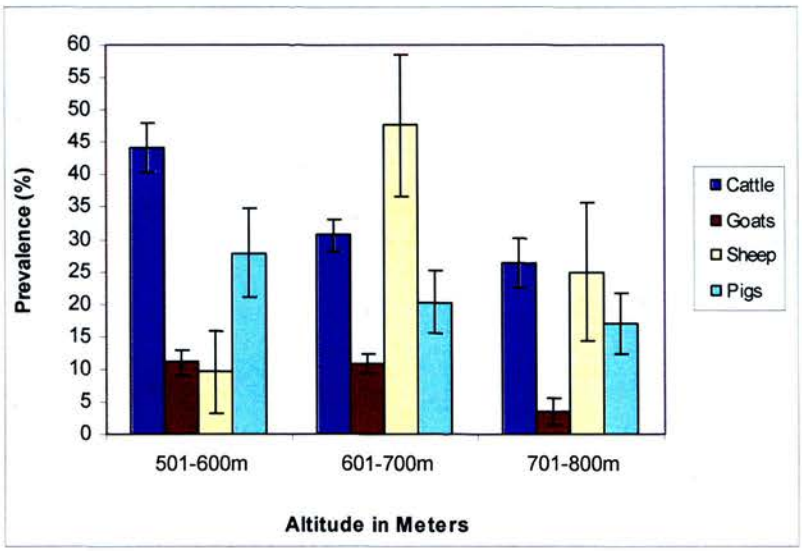
Although the proportion of the pigs infected showed that more infection were detected at low altitude at the 0.05% level of significance, a linear trend could not be inferred between the proportion of affected pigs and the altitude (χ^2 = 1.800, d.f. = 1, *p*-value=0.179). Sheep did not show any trend at all (χ^2 =1.563, df = 1, *p*-value=0.211).

Table 4. 7 Trypanosomiasis prevalence in livestock at different altitude

	Cattle			Goats			Sheep			Pigs		
Altitude	Number sampled	Nº +ve	Prev. (%)	Number sampled	Nº +ve	Prev. (%)	Number sampled	Nº +ve	Prev. (%)	Number Sampled	Nº +ve	Prev. (%)
450-500	-	-	-	-	-	-	-	-	-	-	-	-
501-600	170	75	44.2	288	32	11.1	21	2	9.52	43	12	27.9
601-700	342	105	30.7	439	48	10.9	21	10	47.6	69	14	20.3
701-800	137	36	26.3	84	3	3.57	16	4	25.0	65	11	16.9
Total	649	216	33.3	811	83	10.2	58	16	27.6	177	37	20.9

Prev. = prevalence, Nº +ve = Number of animals positive

Figure 4. 4 Prevalence in livestock at different altitude



4.4.5. Species of trypanosomes by altitude in cattle

In cattle there was no distinct pattern of distribution that the species of *T. congolense (*savannah type*)* followed from low to high altitude. A pattern was observed with *T. vivax* were it seemed to have been decreasing with altitude (Table 4.8). Generally prevalence of *T. vivax* was higher at low altitude and this decreased as the altitude increased. Beyond 750 meters above see level no *T. vivax* species were detected. The difference between the proportion of *T. vivax* found in the areas below 750 meters (34/581 = 5.85%) sea level and above this level (0/68 = 0.00%) was significant at 0.05% level (*p*-value = 0.039).

Table 4. 8 Species of trypanosomes by altitude in cattle

Altitude (m)	*Tc*	*Tv*	*Tb*	*Tc/Tv*	*Tc/Tv/Tb*	No of animals sampled
551-600	50 (29.4%)	14 (8.24%)	1 (0.59%)	7 (4.12%)	2 (1.18%)	170
601-650	30 (19.35%)	8 (5.16%)	0 (0.00%)	2 (1.29%)	0 (0.00%)	155
651-700	47 (25.1%)	6 (3.21%)	3 (1.60%)	7 (3.74%)	0 (0.00%)	187
701-750	18 (26.0%)	6 (8.70%)	0 (0.00%)	1 (1.45%)	0 (0.00%)	69
751-800	13 (20.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	65
801-850	1 (33.33%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	3
						649

Households keeping cattle starts at 559 meters above sea level

4.4.6. Trypanosomiasis prevalence in livestock based on size of households

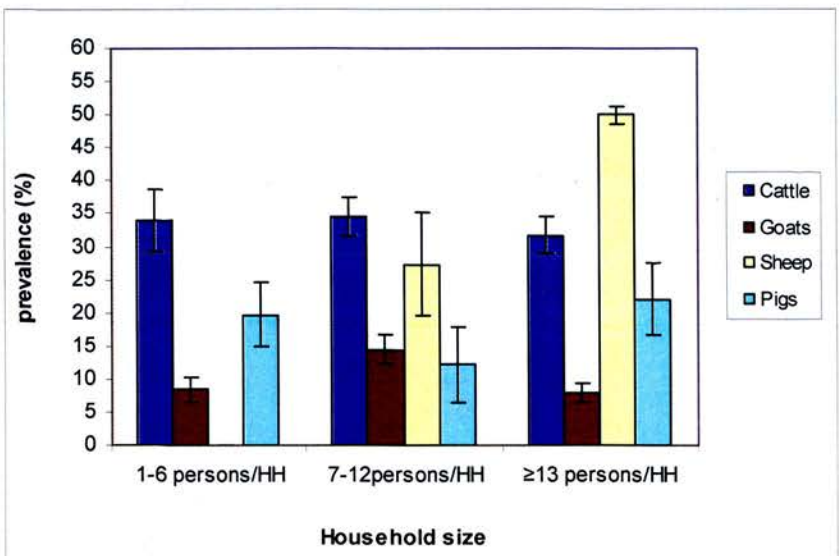
In cattle, goats and pigs, there was no clear defined pattern of the disease prevalence based on the size of the households (Table 4.9 and Figure 4.5). A clear distinct pattern was only seen in sheep where the disease prevalence increased with the size of the households. In sheep, at the 0.05% level of significance, a linear trend could be inferred between the proportion of affected sheep and the household size ($\chi^2 = 7.676$, d.f. = 1, *p*-value=0.005). In the households with a family size of 1-6persons/household no sheep was infected 0/11(0%), in households with 7-12 persons/HH 9/33 sheep (27.3%) were infected and in the households of the family size of ≥13, 7/14 (50%) sheep were infected (Figure 4.5).

Table 4. 9. Trypanosomiasis presence in livestock based on size of household size

	1-6 person/HH			7-12 person/HH			≥13 person/HH		
Livestock species	Sample	No of positive	%	Sample	No of positive	%	Sample	No of positive	%
Cattle	102	35	34	257	89	34.6	290	92	31.7
Goats	227	19	8.37	270	39	14.4	316	25	7.91
Sheep	11	0	0	33	9	27.3	14	7	50.0
Pigs	66	13	19.70	52	11	12.2	59	13	22.0

HH= Households

Figure 4. 5 Trypanosomiasis presence in livestock based on size of household



4.4.7. Species of trypanosomes in the livestock in the area

4.4.7.1. Species of trypanosomes

The proportions of the species of trypanosomes as determined by microscopy in cattle were 88.0% (*T. congolense*), 10.4% (*T. vivax*), 1.6% (*T. brucei*) and by PCR was 73.7% (*T. congolense*), 23.2% (*T. vivax*), 2% (*T. brucei*) and 1% (*T. theileri*), (Tables 4.11a, b,

c, d and e). The ratio of microscopy to PCR were, *T. congolense* (1:1), *T. vivax* (1:2), *T. brucei* (1:2). The difference in proportion detected by microscopy and PCR were significant at the 0.05% level for *T. congolense* (χ^2 =9.44, d.f. = 1, *p*-value = 0.004) and *T. vivax* (χ^2 = 8.28, d.f. = 1, *p*-value = 0.004). For *T. brucei* it was not significant (*p*-value = 0.419).

In goats trypanosome species as determined by microscopy were 88.6% (*T. congolense*) and 11.4% (*T. vivax*). By PCR they were 57.69% (*T. congolense*) and 42.3% (*T. vivax*). The ratio of microscopy to PCR were, *T. congolense* (1:1) and *T. vivax* (1:4). The difference in prevalence in microscopy to PCR were significant at the 0.05% level for both *T. congolense* and *T. vivax* (χ^2 =10.877, df = 1, *p*-value = 0.001). No *T. brucei* was detected by both methods in goats (Tables 4.10a, b, c, d and e).

The prevalence of the species of trypanosomiasis in pigs by microscopy was 93.8% (*T. congolense*) and 6.25% (*T. brucei*). By PCR it was 41.4% (*T. congolense*), 17.2% (*T. vivax*), 6.90% (*T. brucei*) and 34.5% (*T. simiae*). The difference in the species prevalence between microscopy and PCR was significant at the 0.05% level for both *T. congolense* (P < 0.05, *p*-value = 0.0005) and not significant for *T. brucei* (P > 0.05, *p*-value = 1). The ratio of microscopy to PCR were, *T. congolense* (2:1) and *T. brucei* (1:1). *T. simiae, T. vivax* and the non pathogenic *T. theileri* were detected only by PCR (Tables 4.10a, b, c, d and e).

In sheep microscopy detected only *T. congolense* (100%). PCR detected *T. congolense* (81.2%) and *T. vivax* (18.8%). The ratio of microscopy to PCR were, *T. congolense* (1:1). The difference in the species prevalence between microscopy and PCR were not significant at the 0.05% level for *T. congolense* (P > 0.05, *p*-value = 0.549), (Tables 4.10a, b, c, d and e).

Table 4. 10 Species of trypanosomes in cattle, goats, pigs and sheep.

A. Species in cattle

Spp	T&T[1]	%	PCR	%
Tc	110	88	140	73.7
Tv	13	10.4	44	23.2
Tb	2	1.6	4	2.10
Ts	0	0	0	0
Tt	0	0	2	1.05
	125		190	

1 = Thick and thin Giemsa stained blood films.

B. Species in Pigs

Spp	T&T	%	PCR	%
Tc	15	93.8	12	41.4
Tv	0	0	5	17.2
Tb	1	6.2	2	6.90
Ts	-	-	10	34.5
Tt	0	0	0	0
	16		29	

C. Species in Goats

Spp	T&T	%	PCR	%
Tc	39	88.6	31	57.7
Tv	5	11.4	22	42.3
Tb	0		0	0
Ts	0		0	0
Tt	0		0	0
	44		53	

D. Species in Sheep

Spp	T&T	%	PCR	%
Tc	5	100	13	81.25
Tv	0	0	3	18.75
Tb	0	0	0	0
Ts	0	0	0	0
Tt	0	0	0	0
	5		16	

E. Trypanosome species in different host animals combined Microscopy and PCR results

		Trypanosoma species							
Animal	Number Tryps+ve	*Tc*	*Tv*	*Tb*	*Ts*	*Tc/Tv*	*Tc/Tv/Tb*	*Tv/Ts*	*Tb/Ts*
Cattle	216	159 (73.6%)	34 (15.7%)	4 (1.85%)	0 (0%)	17 (7.87%)	2 (0.92%)	0 (0%)	0 (0%)
Goats	83	57 (68.7%)	21 (25.3)	0 (0%)	0 (0%)	5 (6.02%)	0 (0%)	0 (0%)	0 (0%)
Sheep	16	13 (81.2%)	3 (18.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pigs	37	23 (62.2%)	2 (5.7%)	1 (2.86%)	8 (22.9%)	1 (2.86%)	0 (0%)	1 (2.86%)	1 (2.86%)

Tryps+ve = trypanosome positive

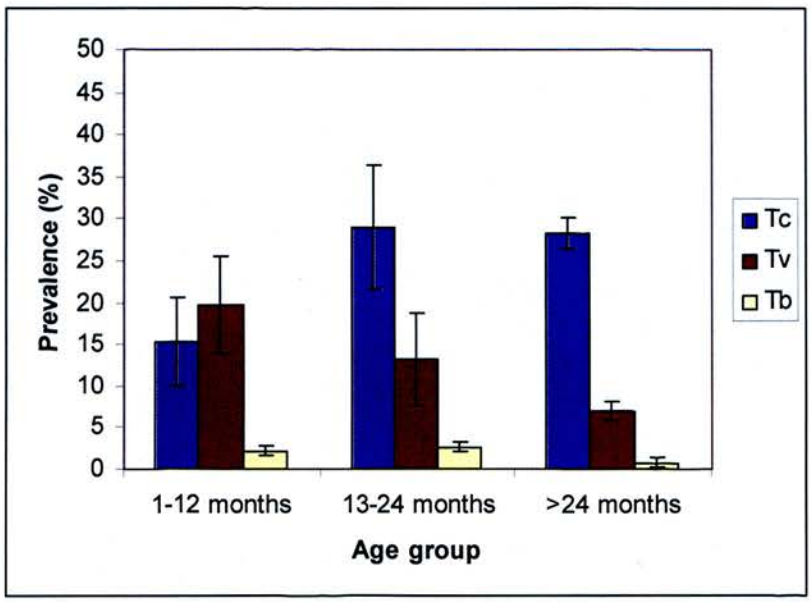
4.4.7.2. Species of trypanosomes by age group of livestock

In cattle the prevalence of *T. congolense* increased with age of the animals. The prevalence was high in the animals in the age groups of 13-24 months and >24 months and lowest in the age group of 1-12 months. Since groups 13-24 months and >24 months were similar they were combined and a linear trend analysed, resulting in a significant result at 0.05% level of significance ($\chi^2 = 3.708$, d.f. = 1, p-value=0.054). The prevalence of *T. vivax* decreased with the increase in the age of animals and this was highly significant at 0.05% level of significance ($\chi^2 = 10.435$, d.f. = 1, $p = 0.001$) The prevalence of *T. brucei* could not be followed properly as they were a few in the area (Table 4.11 and Figure 4.6).

Table 4. 11 Species of trypanosomes by age group in cattle

	1-12 months			13-24 months			>24 months		
	Number of animals sampled	**Animals Positive**	**%**	**Number of animals sampled**	**Animals Positive**	**%**	**Number of animals sampled**	**Animals Positive**	**%**
Tc	46	7	15.2	38	11	28.9	565	160	28.3
Tv	46	9	19.6	38	5	13.2	565	39	6.90
Tb	46	1	2.17	38	1	2.63	565	4	0.71

Figure 4. 6 Species of trypanosomes by age group in cattle

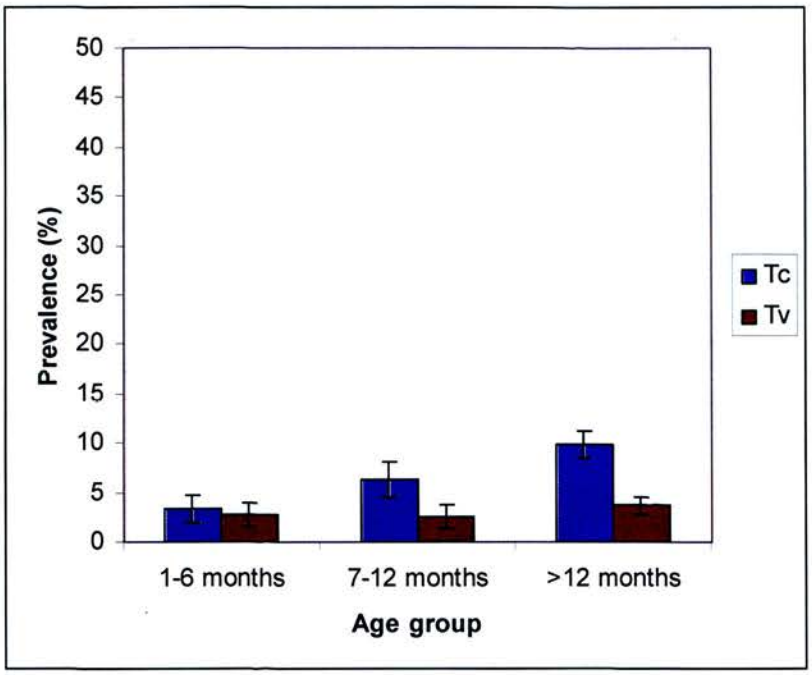


In goats the prevalence of *T. congolense* and *T. vivax* increased with age of the animals. The prevalence was also high in the animals in the age group of >12 months and lowest in the age group of 1-6 months (Table 4.12 and Figure 4.7). There was a linear trend between the proportion of *T. congolense* and the age groups which was significant at 0.05% level of significance ($\chi^2 = 25.723$, d.f. =1, $p < 0.001$). There was also a linear trend in *T. vivax* prevalence in the age groups and was significant ($\chi^2 = 3.885$, d.f. = 1, p= 0.049)

Table 4. 12 Species of trypanosomes by age group in goats

	1-6 months			7-12 months			>12 months		
	Number of animals sampled	Animals Positive	%	Number of animals sampled	Animals Positive	%	Number of animals sampled	Animals Positive	%
Tc	184	7	3.80	191	12	6.28	436	43	9.86
Tv	184	5	2.72	191	5	2.62	436	16	3.67

Figure 4. 7 Species of trypanosomes by age group in goats

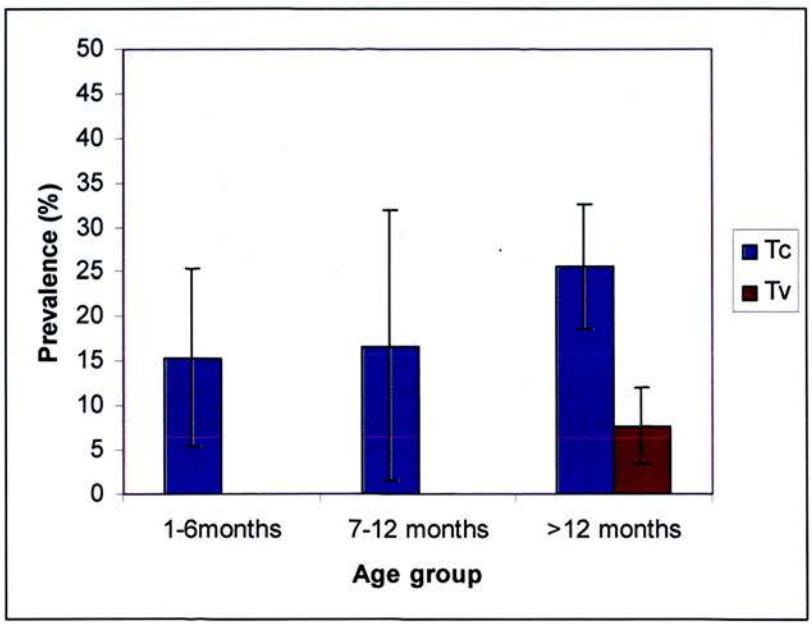


Although the proportion of *T. congolense* increased with the age in the sheep (Table 4.13 and Figure 4.8), this was not significant at 0.05% significance level ($\chi^2 = 0.6727$, d.f. = 1, *p*-value = 0.4121). Even if the age groups of 1-6 months and 7-12months are combined since the prevalence in this two groups are not significantly different a linear trend is still not significant at 0.05% significant level ($\chi^2 = 1.6181$, d.f. = 1, *p*-value = 0.2034). *T. vivax* infection was detected only in the age group of >20 months.

Table 4. 13 Species of trypanosomes by age group in sheep

	1-6months			7-12 months			>12 months		
	Number of animals sampled	**Animals Positive**	**%**	**Number of animals sampled**	**Animals Positive**	**%**	**Number of animals sampled**	Animals Positive	%
Tc	13	2	15.4	6	1	16.7	39	10	25.6
Tv	13	0	0.00	6	0	0.00	39	3	7.69

Figure 4. 8 Species of trypanosomes by age group in sheep

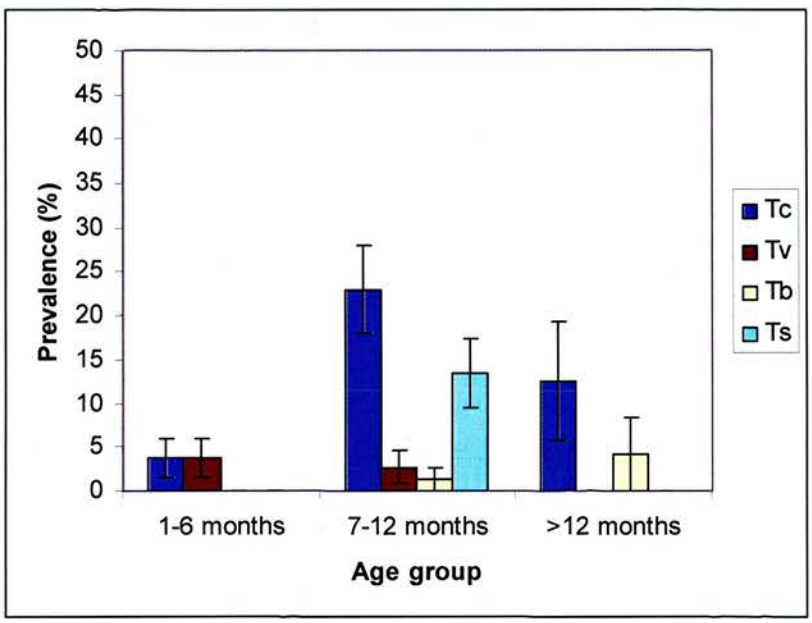


In the pigs no linear trend in any trypanosome species was observed (Table 4.14 and Figure 4.9). All the *T. simiae* infections were detected in the age group of 7-12 months and most of the *T. congolense* (23.0%) were detected in the age group of 7-12 months. Only two *T. brucei* were detected, one in the age group of 7-12 months and the other one in the age group of >12 months. *T. vivax* were detected only in the younger groups of 1-6 months and 7-12 months.

Table 4. 14 Species of trypanosomes by age group in pigs

	1-6 months			7-12 months			>12 months		
	No	pos	%	No	pos	%	No	pos	%
Tc	79	3	3.79	74	17	23.0	24	3	12.5
Tv	79	3	3.79	74	2	2.70	24	0	0.00
Tb	79	0	0.00	74	1	1.35	24	1	4.17
Ts	79	0	0.00	74	10	13.5	24	0	0.00

Figure 4. 9 Species of trypanosomes by age group in pigs



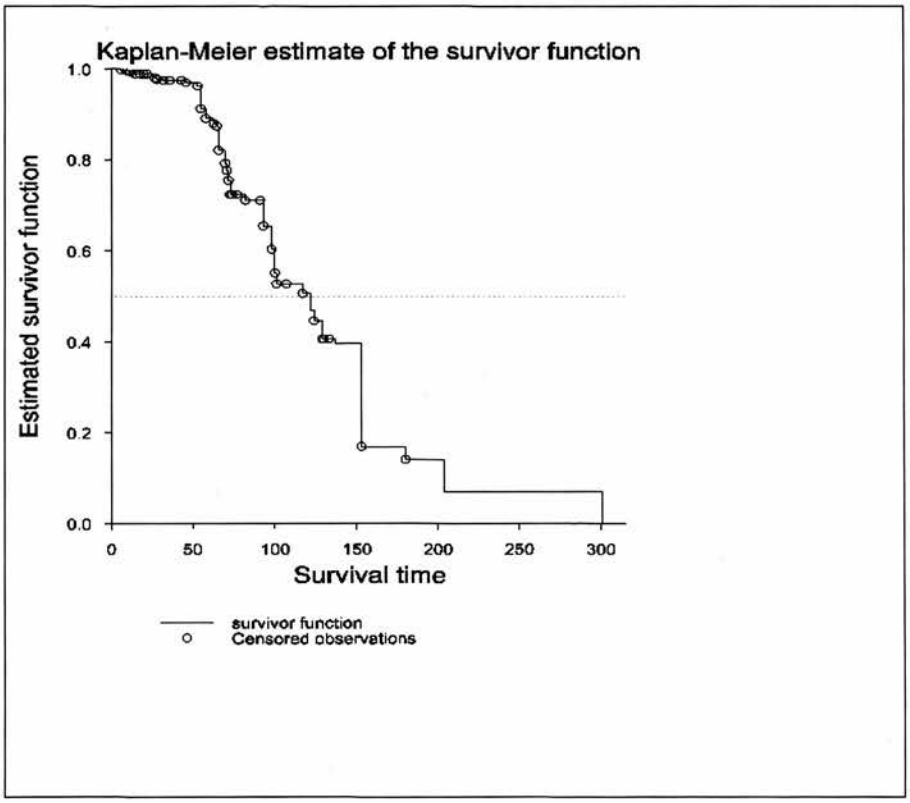
4.4.8. Trypanosomiasis prevalence in treated cattle

4.4.8.1. Cattle treated with isometamidium chloride (ISMM)

Data were analysed to see how fast cattle became positive after they were treated with isometamidium chloride by farmers and local veterinary officials. In total 566 cattle were treated with isometamidium chloride, 46 with diminazene aceturate and only 37 were not treated in the last six months. In all 25% of the cattle became infected by 73 days, 50%

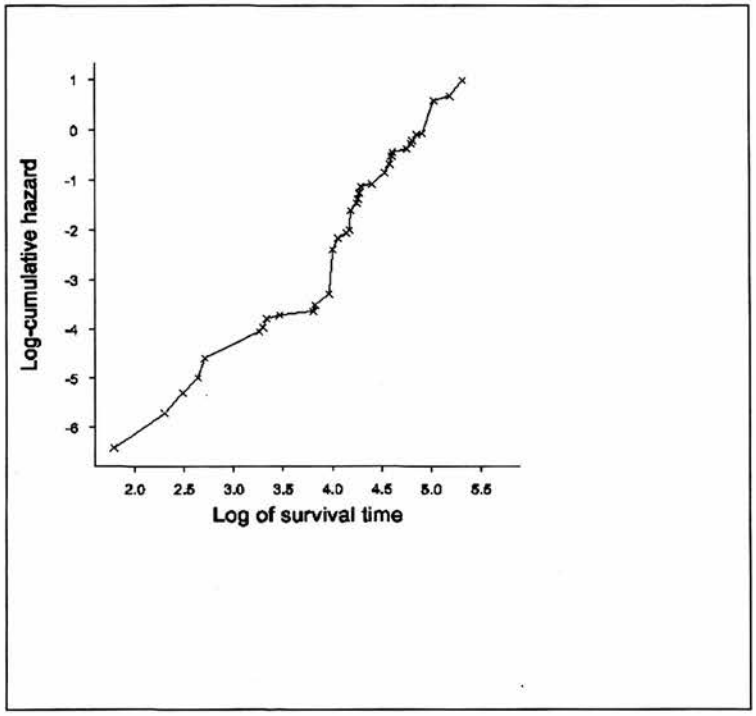
by 122 days, and 75% by 153 days. This means the median survival time (time until half the cattle become infected) was 122 days (95%CI: 100 to 129) About 18.5% (5/27) of the cattle that were treated with isometamidium chloride less than a month before the sampling date were found to be positive for trypanosomes. In the group that were treated less than three months 39.6% (38/96) of the cattle were positive for trypanosomes. By the fourth month about 50% (16/32) were positive for the parasite (Table 4.15 and Figures 4.10, 4.11 and 4.12). About 36.84% (7/19) of cattle that were treated with diminazene aceturate became positive for trypanosomes after a month of been treated by the farmers (Table 4.16). Figure 4.10 shows the Kaplan- Meier survival (time to infection) curve for animals that were treated with isometamidium chloride.

Figure 4. 10 Estimated survivor function



This graph shows the survival time i.e. the time the animals took to become positive from the time they were treated with isometamidium chloride by the farmers and veterinary officials. X-axis indicating time in days and Y-axis showing the proportion of animals becoming positive.

Figure 4. 11 Log-cumulative hazard



This graph shows the survival time i.e. the time the animals took to become positive from the time they were treated with isometamidium chloride by the farmers and veterinary officials. This graph is similar to figure 4.10 but this is in log scale.

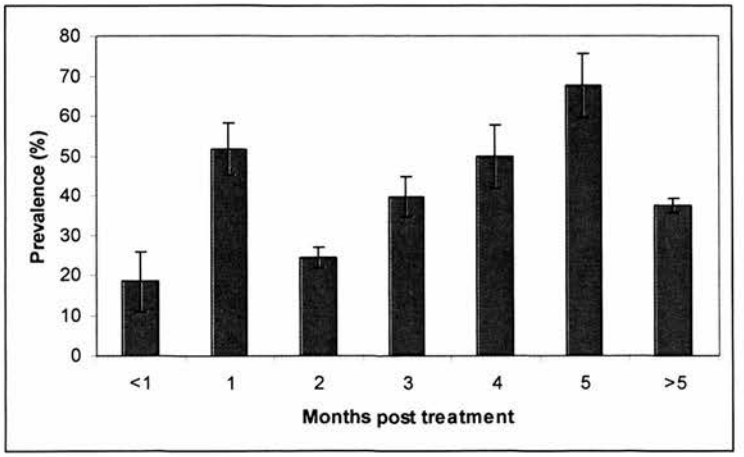
Table 4. 15 Prevalence of trypanosomiasis in cattle on the basis of how recently they were reported to have been treated with isometamidium chloride

Species of trypanosomes	<1	1	2	3	4	5	>5
Tc	5 (100%)	24 (80%)	54 (71.05%)	30 (78.95%)	11 (68.75)	14 (60.87%	3 (100%)
Tv	0	2 (6.67%)	15 (19.74%)	3 (7.89%	3 (18.75%	5 (21.74%	0
Tb	0	1 (3.33%)	0	0	0	1 (4.35%)	0
Tc/Tv	0	3 (10%)	6 (7.89%)	5 (13.56%)	2 (12.5%)	2 (18.18%)	0
Tc/Tv/Tb	0	0	1 (1.32%)	0	0	1 (4.35%)	0
Number of positive animals (all species)	5	30	76	38	16	23	3
Percentage (%) positive	18.5	51.7	24.4	39.6	50	67.6	37.5
Total number of animals	27	58	311	96	32	34	8

Table 4. 16 Species of trypanosomes in re-infected animals post treatment with diminezene aceturate.

	Months post treatment		
Species of trypanosomes	<1	1	>1
Tc	1 (100%)	6 (85.7%)	4 (80%)
Tv	0	0	1 (20.0%)
Tb	0	0	0
Tc/Tv	0	1 (14.28%)	0
Tc/Tv/Tb	0	0	0
Number of positive animals	1	7	5
Percentage (%) positive	8.33	36.8	33.3
Total number of animals	12	19	15

Figure 4. 12 Prevalence of trypanosomiasis in cattle on the basis of how recently they were reported to have been treated with isometamidium chloride



4.4.8.2. Species of trypanosomes infecting cattle recently treated with isometamidium chloride and diminezene aceturate by farmers.

Cattle that were recently treated were infected more with *T. congolense* than any other trypanosome species. In those animals that were infected less than a month after being treated with isometamidium by the farmers all (5/5) of the trypanosomes were *T. congolense* (Table 4.16). In 30 animals that were infected one month after they were treated with isometamidium chloride, 24 (80%) were *T. congolense*, 2 (67%) were *T. vivax*, 1 (3.33%) was *T. brucei* and 3 (10%) were mixed infection of *T. congolense/T. vivax*. Similar ratios of trypanosome species were observed in cattle treated between 2-5 months earlier. Infection with *T. brucei* either alone or in mixed infection was rarely observed in recently treated cattle. The infections between *T. c*ongolense and other species was significant.

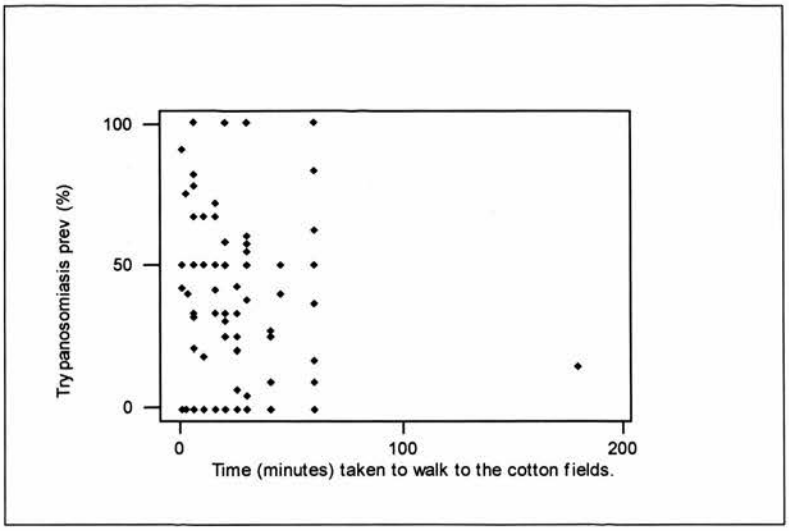
One month post-treatment with ISSM	$= \chi^2 = 31.188$, d.f. is 3, p-value < 0.001
Two months post-treatment with ISSM	$= \chi^2 = 78.872$, d.f. is 3, p-value < 0.001
Three months post-treatment with ISSM	$= \chi^2 = 26.920$, d.f. is 2, p-value < 0.001
Four months post-treatment with ISSM	$= p$-value $= 0.0597$
Five months post-treatment with ISSM	$= p$-value $= 0.0058$

Most infections in animals recently treated with diminezene aceturate were *T. congolense* (11 (84.6%)). Only 1 (7.69%) was *T. vivax* and one was a mixed infection of *Tv/Tc* (1 = 7.69%).

4.4.9. Effect of cotton growing on trypanosomiasis prevalence in cattle

It is hypothesied that trypanosomiasis prevalence in animals is low in households that are near cotton fields compared with those housholds located far from the fields. There was no significant association between the prevalence and the time (Figure 4.13) the farmers take to walk from their homes to the cotton fields (r = -0.112 and *p*-value = 0.278).

Figure 4. 13 Trypanosomiasis prevalence (%) against time taken to walk to the cotton fields.



4.4.10. Haemoglobin values for Livestock in Mambwe District

4.4.10.1. Haemoglobin values in different species of animals

The mean haemoglobin value (Table 4.17a) for the cattle was 9.61g/dl, goats 10.92g/dl, sheep 10.35g/dl and pig 12.97g/dl. The mean haemoglobin value was higher in pigs and lowest in cattle.

Table 4. 17a Mean Haemoglobin (g/dl) for livestock species

	Mean	Max	Min	Median	StDev	n
Cattle	9.61	15.50	3.60	9.60	2.14	649
Goats	10.92	16.30	3.70	10.20	1.99	811
Sheep	10.34	16.60	3.00	11.10	2.92	57
Pigs	12.97	17.90	2.70	12.95	2.27	174

n = number of animals

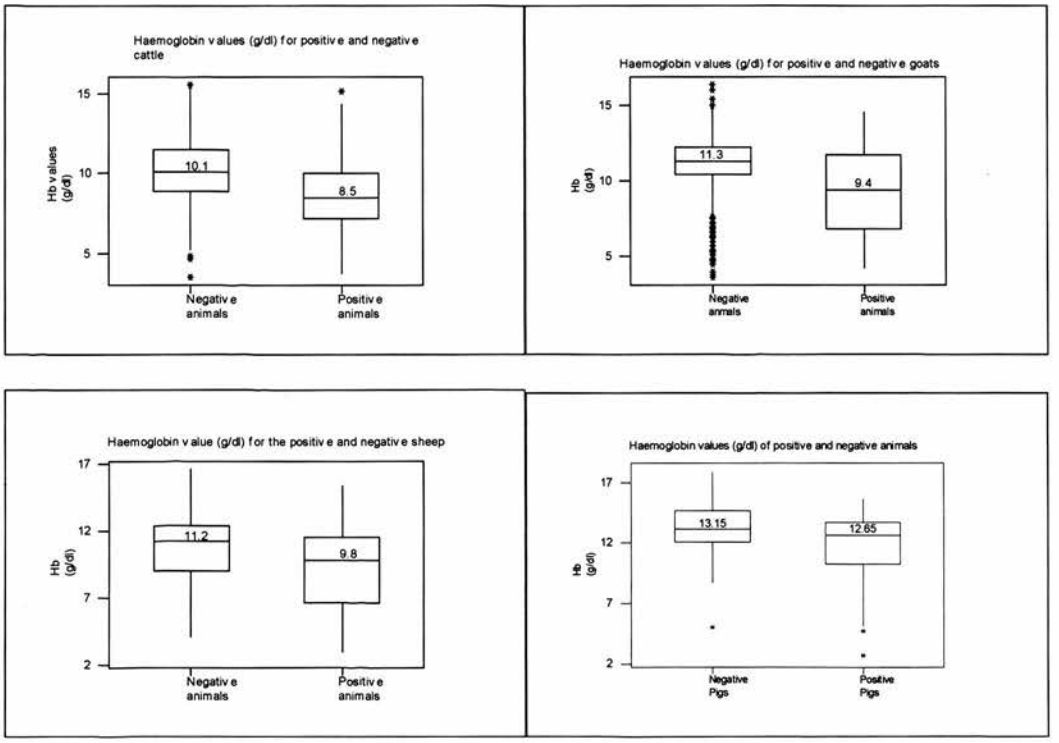
The mean haemoglobin value (Table 4.17b and Figure 4.14) for the cattle (8.53 g/dl ± 2.08 g/dl) positive for trypanosomes was significantly lower (t-test, $p < 0.001$) than that of the animals that were negative (10.15 g/dl ± 1.96 g/dl). In goats the mean value for the positive animals (9.13g/dl ± 2.64 g/dl) was lower than that of the negative ones (11.13g/dl ± 1.79 g/dl) and was significantly different (t-test, $p < 0.001$). The same was observed for the positive sheep (9.13g/dl ± 3.62g/dl) that was significantly lower (t-test, p < 0.001) than that of the negative sheep (10.73 ± 2.54g/dl). Although the mean haemoglobin values for positive pigs (11.71g/dl ± 3.18g/dl) was lower than that for the negative pigs (13.28 ± 1.88g/dl) it was not significantly different (t-test, $p > 0.001$).

Table 4. 17b Mean Haemoglobin (g/dl) for Livestock positive and negative for trypanosomes

	Mean	Max	Min	Median	StDev	n
Cattle						
1. Positive	8.53	15.1	3.80	8.50	2.08	216
2. Negative	10.2	15.5	3.60	10.1	1.96	433
Goats						
1. Positive	9.13	14.5	4.30	9.40	2.64	83
2. Negative	11.1	16.3	3.70	11.3	1.79	728
Sheep						
1. Positive	9.36	15.4	3.00	9.80	3.62	16
2. Negative	10.73	16.6	4.10	11.2	2.54	41
Pigs						
1. Positive	11.71	15.7	2.70	12.6	3.18	34
2. Negative	13.28	17.9	5.00	13.2	1.88	140

n = number of animals

Figure 4. 14 Haemoglobin values for Livestock (cattle, goats, sheep and pigs)



75th centile (upper quartile) = 75% of the observations lie below and 25% above this value.

Median (50th centile or quantile) = value below which half, and above which half, of the observations lie.

25th centile (lower quartile) = 75% of the observations lie above it and 25% below this value. (M. Thrusfield, 2000)

***** = Outliers**

4.4.10.2. Haemoglobin values in livestock infected by different species of trypanosomes

4.4.10.2.1. The mean haemoglobin values for the cattle

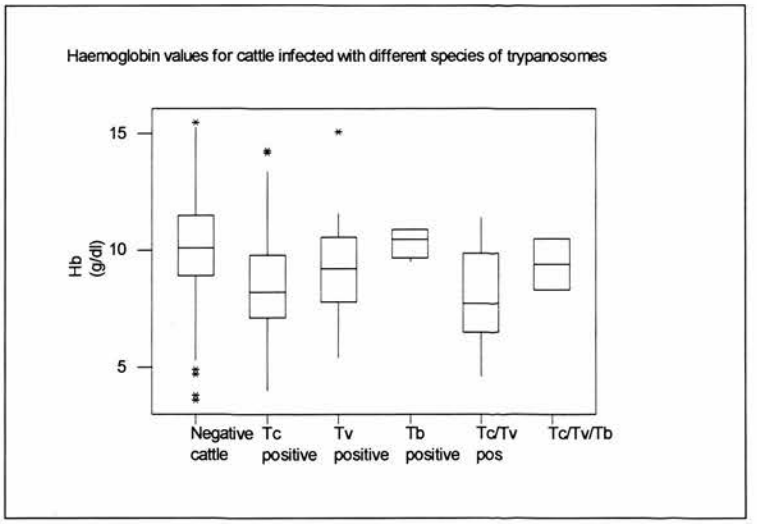
The mean haemoglobin values were lowest in the group infected with a mixed infection of *T. congolense* and *T. vivax*. This was followed by cattle infected with *T. congolense* only and then *T. vivax* only. The difference in the mean haemoglobin values of these three groups and the mean of group that was negative were statistically significant (t-test; $p < 0.05$). The two groups that had high mean haemoglobin values were those infected with *T.* brucei followed by the group that had mixed infection of *Tc/Tv/Tb.* The mean haemoglobin values of these groups were not different statistically from the mean of negative animals (Table 4.18 and Figure 4.15).

Table 4. 18 The mean haemoglobin values for the cattle infected with different species of trypanosomes.

Group	Number of samples	Mean	StDev	95%CI of difference	T-value	DF	P-value	Sig. diff.
Negative	433	10.12	1.99					
Tc	159	8.45	2.10	1.303 to 2.038	8.92	590	<0.001	Yes
Tv	34	9.32	1.90	0.099 to 1.487	2.25	465	0.025	Yes
Tb	4	10.32	0.66	-2.166 to 1.749	-0.21	435	0.834	No
Tc/Tv	17	8.05	2.01	1.006 to 2.838	4.12	450	<0.001	Yes
Tc/Tv/Tb	2	9.40	1.56	-2.05 to 3.49	0.51	433	0.611	No

StDev = standard deviation, DF = degrees of freedom, Sig. diff = Significant different.

Figure 4. 15 The mean haemoglobin values for cattle



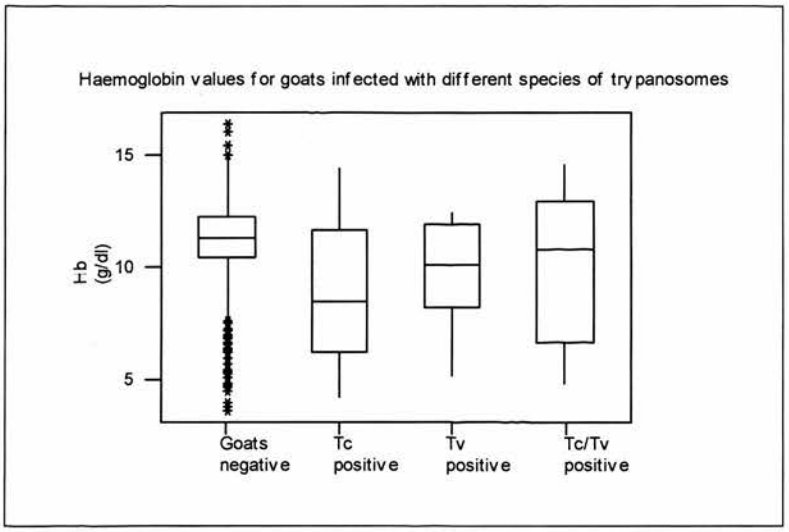
4.4.10.2.2. Mean haemoglobin values for the goats

The mean haemoglobin value was lowest in goats infected with *T. congolense* followed by the group of animals that were infected with *T. vivax* infection and these values were lower than the mean of the animals that were negative. The differences were statistically significant (t-test; $p < 0.05$). The mean haemoglobin value for the few animals that were infected with mixed infection of *Tv/Tc* did not differ from the mean of the animals that were negative (t-test; $p > 0.05$.) (Table 4.19 and Figure 4.16). Out of the five mixed infection detected, four where diagnosed by PCR and only one was by microscopy.

Table 4.19 The mean haemoglobin values for the goats infected with different species of trypanosomes.

Group	Number of samples	Mean	StDev	95%CI of difference	T-value	DF	P-value	Sig. diff.
Negative	728	11.13	1.79					
Tc	57	8.80	2.70	1.824 to 2.835	9.05	783	<0.001	Yes
Tv	21	9.81	2.14	0.535 to 2.102	3.31	747	0.001	Yes
Tc/Tv	5	10.00	3.58	-0.464 to 2.720	1.39	731	0.165	No

Figure 4. 16 The mean haemoglobin values for the goats infected with different species of trypanosomes.



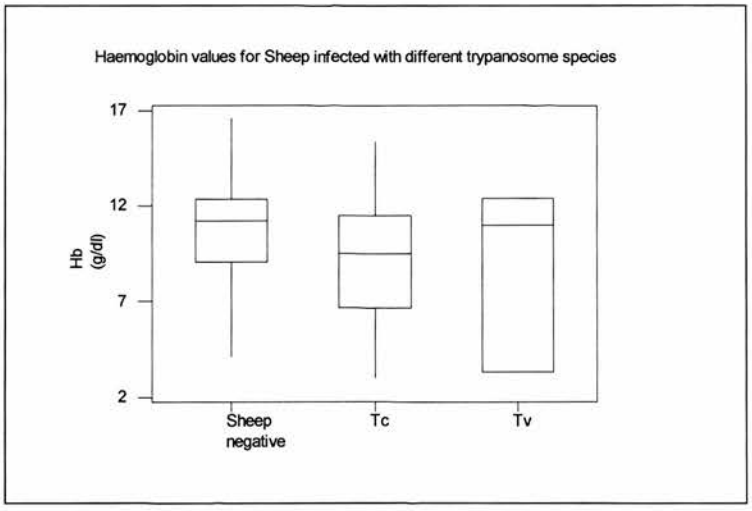
4.4.10.2.3. The mean haemoglobin values for the sheep infected with different species of trypanosomes.

The mean haemoglobin values (Table 4.20 and Figure 4.17) of the sheep infected with different trypanosomes species were not significantly different from the mean haemoglobin values of the negative animals (t-test; $p > 0.05$).

Table 4. 20 The mean haemoglobin values for the sheep infected with different species of trypanosomes.

Group	Number of samples	Mean	StDev	95%CI of difference	T-value	P-value	DF	Sig. diff.
Negative	41	10.73						
Tc	13	9.47		-0.522 to 3.047	1.42	0.162	52	No
Tv	3	8.90		-1.43 to 5.09	1.13	0.263	42	No

Figure 4. 17 The mean haemoglobin values for the sheep infected with different species of trypanosomes.



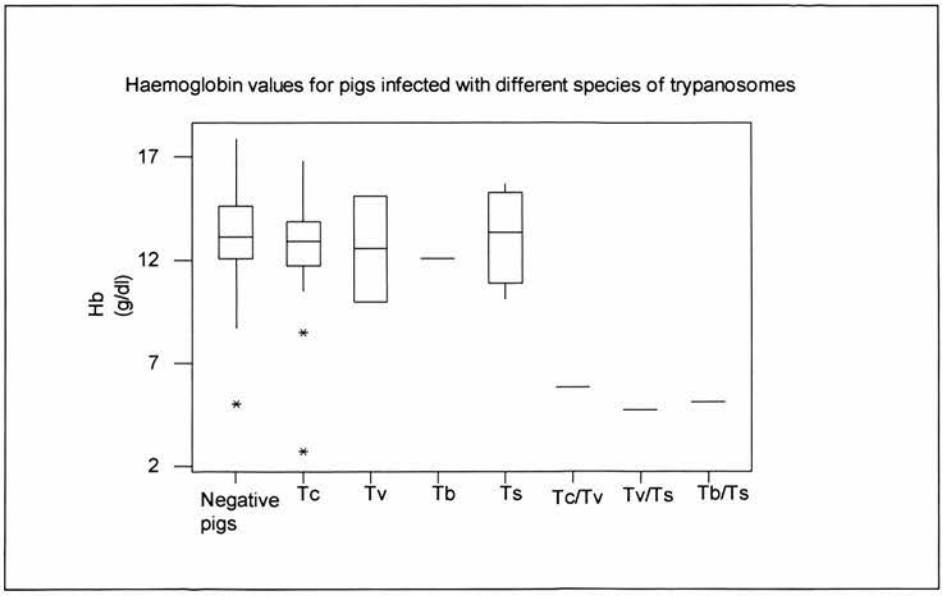
4.4.10.2.4. The mean haemoglobin values for the pigs infected with different species of trypanosomes.

The mean haemoglobin values of the pigs that were infected with single infection of trypansomes species of either *T. congolense* or *T.vivax* or *T.brucei* or *T. simiae* (Table 4.21 and Figure 4.18) were not statistically different from the mean of the animals that were negative for trypanosomes (t-test; $p > 0.05$). The pigs that were infected with mixed infection showed much lower haemoglobin values than those that were infected with single species trypanosomes and those that were negative (Table 4.21 and Figure 4.18).

Table 4. 21 The mean haemoglobin values for the pigs infected with different species of trypanosomes.

Group	Number of samples	Mean	StDev	95%CI of difference	T-value	P-value	DF	Sig. diff.
Negative	139	13.21	1.88					
Tc	22	12.36	2.86	-0.042 to 1.792	1.89	0.061	158	No
Tv	2	12.55	3.61	-1.96 to 3.34	0.51	0.607	138	No
Tb	1	12.10	0	Analysis not possible - few samples				
Ts	8	13.21	2.14	-1.321, 1.375	0.04	0.969	144	No
Tc/Tv	1	5.80	0	Analysis not possible - few samples				
Tv/Ts	1	4.70	0	"				
Tb/Ts	1	5.10	0	"				

Figure 4. 18 The mean haemoglobin values for the pigs infected with different species of trypanosomes.



4.5. Discussion

4.5.1. Trypanosomiasis prevalence pattern in the households keeping different combination of different livestock

The results of this work clearly defined animal trypanosomiasis prevalence pattern in the households keeping a combination of different livestock kept in the area. The prevalence has been determined in animals kept as individual species and where different combinations of these species of livestock are kept. The overall trypanosomiasis prevalence regardless of the combination of animal species kept, showed that disease prevalence in the area was highest in cattle (33.3%) followed by sheep (27.6%), pigs (20.90%) and lastly goats (10.2%). The order followed in this case was cattle > sheep > pigs > goats. The higher prevalence of infection detected in sheep clearly demonstrated that even small ruminants do get infected with trypanosomes. Similar results have also been demonstrated in Kenya (Ng'ayo *et al*, 2005).

When a combination of domestic livestock kept was taken into consideration, in households keeping individual species of livestock, trypanosomiasis prevalence was high in cattle (28.4%) followed by pigs (21.5%). This was followed by sheep (18.2%) and the least affected were goats (9.22%). The pattern of prevalence was cattle>pigs>sheep>goats. Livestock kept in different combination followed the same pattern with a few variations (though not significant) in households where only cattle and pigs were kept. In this case the prevalence was higher in pigs (26.7%) and low in cattle (23.1%). The prevalence pattern followed in this case was; Pigs > Cattle. This demonstrates that cattle are more prone to infection than the other species that were studied in this area. This seems to follow the feeding preference of the tsetse flies in the area and is in agreement with the survey that was done on the feeding habits of *Glossina morsitans morsitans* in the adjacent area in Katete District (Van den Bossche, 1997) where cattle were the main host of *Glossina morsitans morsitans*. The possible explanation for the variation in some areas where the prevalence was high in pigs than in cattle could be that this area where the study was conducted lies in the Luangwa valley where it has been reported that *Glossina morsitans morsitans* take majority of their blood meals from wild suidae (Sehof, 1975). Elsewhere it has been reported that *Glossina*

pallidipes which is also present in the area feed mainly on ruminants (buffalo, bushbuck and cattle) but depending on host availability and location, suidae also provided blood meal (Clausen, 1998). Another explanation for the observation in this study is that larger host animals or more of them, are more attractive to tsetse than smaller host animals because of the large amount of odour produced (Torr *et al.*, 2006; Simukoko *et al*, 2007).. In addition to this, some host animals like goats prevent flies from feeding by twitching the skin (FAO training manual, 1982; Baylis, 1996; Torr and Mangwiro, 2000). Another reason why goats were least affected might be that the Valley goats a type of Small East African goat breed is trypanotolerant (Griffin & Allonby 1979a; Griffin & Allonby 1979b).

4.5.2. Prevalence of trypanosomiasis in livestock species by age group

The proportion of the animals infected with trypanosomes was high in the older groups in all the species. The study revealed that in cattle the prevalence in young animals (1-12 months) was 14/46 (30%), in the middle aged 13/38 (34.2%) and in the age group >24 months the prevalence was 189/565 (33.4%) indicating that calves were less susceptible to trypanosome infection than adults (Fiennes, 1970; Wellde *et al.*, 1981, Murray et al., 1982). The possible explanation to this observation could be the presence of acquired immunity that is playing a part in young animals due to a protective effect of maternal colostrum transferred from mother to offspring with colostrum (Murray et al., 1982). Trypanosomiasis which is a protozoan infection induce milder response in the young ones than in the old (Murray et al., 1982; Trail *et al*, 1994; Rowlands *et al*, 2001). The observation was consistent in goats, sheep and pigs. The other reason could be that the young ones (those that are still suckling) are left behind around the villages when older ones go to graze. This decreases the vector-host contact, hence transmission of the parasites to the young animals as there are few tsetse flies around villages compared to the flies that are found in the bushes where the older animals graze (personal observation). Age seems to play an important role in the epidemiology of the species of the trypanosomes in the area. *T. congolense* prevalence increased with age in cattle, goats, sheep and pigs i.e. it was diagnosed more in older animals than younger ones. The

observation was opposite with *T. vivax* were it was high in the younger animals than the older ones. It is very difficult to tell the course taken by *T. brucei* because of its low prevalence in the area.

4.5.3. Trypanosomiasis prevalence in treated cattle

The results of the animals that were treated with isometamidium chloride by farmers or local veterinary officials showed that 25% of the cattle became infected by 73 days, 50% by 122 days, and 75% by 153 days. This means the median survival time (time until half the cattle become infected) was 122 days (95%CI: 100 to 129) indicating no drug problem. Depending on the challenge (trypanosome risk), isometamidium chloride is known to protect the animals against trypanosomes for a period of two to more than four months (Boyt, 1986). However there is an indication that there is drug treatment problem in the area as 5/27 (18.5%) of the animals that were treated with isometamidium chloride were positive for trypanosomes within 1 month of treatment and 76/311 (24.4%) within 2 months of treatment possibly as a result of wrong drug dilution or underdosing (chapter 3). The sociological survey that we conducted during the same period the trypanosomiasis survey was been done revealed that over 75% of the farmers were administering the drug on their own and most of them did not know how to do it (Chapter 3 of this thesis). This is in contrast with the farmers in the adjacent districts where most of them know how to administer the drug properly (Delespaux *et al*, 2002). Drug resistance can not be ruled out as this has been reported in the adjacent areas of Petauke, Katete and Chipata Districts (Delespeaux *et al*, 2002, Mubanga, 1997, Sinyangwe *et al*, 2004). In the treated animals the species that re-infected them more was *T. congolense*.

4.5.4. Trypanosomiasis prevalence in livestock at different altitude

The results presented on the disease in relationship with altitude show that at low altitude trypanosomiasis prevalence in the animals was high and as the altitude increased the prevalence went down. This effect could be explained in terms of the tsetse fly the vector of the parasites that causes trypanosomiasis. Tsetse thrive very well at 25-26°C and if temperature goes much higher or much lower than this, damage to the fly may be

observed (Hargrove, 1999; Torr & Hargrove, 1999; FAO training manual, 1982). Temperature is dependent on altitude and in this area where the study was conducted temperature is high at low altitude (valley) and as one approaches the plateau (high altitude) it gets cooler (see chapter two, section 2.2.1). Altitude showed some effect on the distribution of *T. vivax* in the area (Table 4.7 and Figure 4.4). More of this species was detected in lower altitude area than in higher ones. The lower areas are near the Luangwa National Park where animal and human interaction is more observed that is to say human and wildlife interface area. This situation has also been observed elsewhere such as in a survey carried out in the Caprivi District of Namibia; more *T. vivax* were diagnosed in areas that were situated near the national park than areas located far away (Van den Bossche, 1999). The possible explanation for this was the abundance of antelopes in such areas. Antelopes are reservoirs of *T. vivax* and tsetse may transmit *T vivax* to them.

Trypanosomiasis prevalence in relationship to the size of households did not reveal any particular pattern in all the four species of the animals sampled.

4.5.5. Species of trypanosomes in the livestock

T. congolense was the most predominant species of trypanosome in all the animals sampled in the area. This species of trypanosomes is the most commonly seen in many wild game species in East and Central Africa and this could be one of the reason why it appeared more commonly than the next most important species, *T. vivax* (Boyt, 1986). Another reason for the persistence of certain species of trypanosome more than the others in the host depends on their virulence. In this area the species of *T. congolense* are more virulent than the *T. vivax* or *T. brucei* species (Masumu *et al.* 2006). Species of trypanosomes that are transmitted more to the host as they develop in tsetse from procyclic to metacyclic forms are the virulent ones (Diffley, 1987; Masumu *et al.* 2006). The virulent, haemorrhagic form of *T. vivax* reported in Kenya has not been recorded in Zambia (Mwongela, 1981). *Trypanosoma brucei* was rarely seen in the animals in the area.

4.5.6. Haemoglobin concentration

Generally the mean haemoglobin values of the infected animals were lower than the mean haemoglobin values for the negative animals. The difference between infected and non-infected animals was seen in cattle. In pigs the mean difference between the infected and the non-infected ones was not significant. The explanation seen in the pigs could be that anaemia, the main sign in the chronic disease as seen in other animals does not develop in most cases, probably because death in pigs as a result of trypanosome infection is so rapid (Boyt, 1986) especially when they are infected with *T. simiae* (Finelle, P. 1983).

Cattle that were infected with *T. congolense/T. vivax* had the lowest mean haemoglobin values and this was followed by those that were infected with single infections of *T. congolense* and *T. vivax* and then *T. vivax/T.congolense/T.brucei.* Cattle that were infected with *T. brucei* did not show any difference from those that were non-infected. This is because infection with *T. brucei* in cattle usually causes mild and almost in-apparent condition. *T. congolense* causes severe anaemia because of its virulence and the chronic nature of the course it follows in cattle (Masumu *et al.* 2006 Bengaly *et al.*, 2002a; Bengaly *et al.*, 2002b).

In sheep all the infections were single infections of either *T. congolense* or *T. vivax* and did not show that they had different effect on the haemoglobin values. In goats *T. congolense* as a single infection had more effect on the haemoglobin values than those animals that were infected with mixed infection probably because most of the mixed infections (4/5) were detected by PCR which is known to detect low levels of trypanosomes in the animal's blood. In this area, most of the goats had very low parasitemia hence less effect on the haemoglobin.

Mixed infections of trypanosomes had more effect on pigs than infections of single trypanosome species. In pigs animals that had mixed infection had very low haemoglobin values. The mean haemoglobin (13.21 g/dl) values of pigs infected with *T. simiae* did not show any difference from that of the non-infected pigs (13.21 g/dl).

4.5.7. Effect of Cotton growing on trypanosomiasis prevalence

Since in this study area most of the houses are situated along the main road and cotton fields situated between the households and the tsetse invasion front, the insecticides (deltamethrin) the farmers are using on cotton might be expected to have a general effect on the trypanosomiasis prevalence in the animals. Unknowingly the farmers create tsetse barriers that protect their animals from tsetse (Muzari, 1999). The deltamethrin a pesticide they use to spray their cotton against pets also kill the tsetse flies (Vale, *et al*, 1999; Mangwiro, *et al*, 1999). The effect of the insecticide was not clearly demonstrated in this study because it was done in the dry season when cotton was been harvested and no spray was taking place. The clearance of land for cotton cultivation also denies tsetse of good habitat. Tsetse need good cool shades for resting and breeding.

4.6. Conclusion

Trypanosomiasis prevalence was inversely related to altitude. At high altitude (approaching the plateau) the vegetation cover was much disturbed than areas that were located on the base of the valley. Trypanosomiasis prevalence was high in lower altitude were vegetation was not much disturbed. The prevalence of trypanosomiasis was high in cattle and low in goats in the area. Generally animals in households keeping a combination of different species of animals had a higher prevalence than those in households where they were keeping only individual animal species. Small ruminants were more likely to be infected if cattle were also present. *T. vivax* infection decreased with age of the animals while that of *T. congolense* increased with age of the animals. The proportion of *T. vivax* was higher in the low altitude areas. Haemoglobin values for the positive animals was much lower in animals that were infected with *T. congolense* than other species. The size of the households did not have any influence on the epidemiology of trypanosomiasis in this study in the area. Cotton growing had a general effect on the trypanosomiasis prevalence in the area.

This study provided further information to the pool of knowledge that already exist on the epidemiology of domestic animal trypanosomiasis in Zambia and in some other places

having similar conditions. Further more this new approach might be a complementary to obtaining information on domestic host-vector interaction that is mainly obtained by identifying the source of the vertebrate blood from the guts of wild-caught tsetse flies (Clausen *et al.*, 1998). This information is important because it will help us in come up with the best way of controlling trypanosomiasis in the area. Currently in the area where this study was conducted control of animal trypanosomiasis is carried out by mass treatment of only cattle with trypanonocidal drugs (isometamidium chloride). In some areas where this study was conducted it was shown that prevalence of trypanosomiasis in pigs (26.7%) was as high as in the cattle (23.1%). In this situation if only cattle are treated by mass treatment trypanosomes will be cleared from them but will remain in pigs that are not treated. Pigs will act as reservoir for these trypanosomes. In this way trypanosomes are maintained and circulate in the animal population in the area as they are not cleared from the pigs and act as source of infection to the cattle.

A single punch from the blood spot could be used for running ITS-PCR as evident from the result obtained from this work. Analysis by ITS-PCR amplification confirmed that it was the most sensitive method for detecting the presence of trypanosomes, some 1.5-fold more sensitive than microscopy. To increase the chance of finding the trypanosomes by microscopy examination, we screened all the fields on the slides. Probably this is why a moderate Kappa value of 0.44 was obtained. The agreement between these two techniques is evidence of validity of the results that were obtained (Thrushfield, 2000). The screening of all the fields on the slides by microscopy was done to make sure no parasite was missed although some were still missed. This microscopy screening demanded many hours of work. Picozzi, *et. al.,* (2002) stated that microscopy is labour intensive and can lack sensitivity under field conditions due to routinely low peripheral parasitaemia in infected livestock. This is exactly what was observed when we were screening animals by microscopy.

5. CHAPTER FIVE

5 PREVALENCE OF TSETSE AND TICK-BORNE DISEASES IN PETAUKE DISTRICT

5.1. Introduction

Tick and tsetse-borne diseases are of major importance to the Zambian livestock industry and are responsible for killing a large number of cattle each year. The most important tick-borne disease in the Eastern Province of Zambia is East Coast Fever (Nambota *et al.* 1994) caused by *Theileria parva,* a parasite transmitted mainly by the ixodid tick *Rhipicephalus appendiculatus,* the three-host African brown ear tick. Tsetse-borne trypanosomiases are caused by protozoan parasites of the genus *Trypanosoma* and spread mainly by the bite of trypanosome-infected tsetse flies. Petauke, the study area is endemic for animal trypanosomiasis and the tick borne-diseases that include East Coast fever, anaplasmosis caused by *Anaplasma* species and babesiosis caused by *Babesia* species. The species of tsetse flies found in the study area in Petauke are *Glossina morsitans morsitans* (Evison and Kathuria, 1982) and the trypanosomes found in livestock are *T. brucei*, *T. congolense* and *T. vivax (*Machila *et al.*, 2001; Sinyangwe. *et al*, 2004*)*.

The objective of the work described in this chapter was to collect baseline data on the prevalence of the parasites (*Anaplasma spp*, *Babesia spp* and *Theileria spp*) that are transmitted by ticks and *trypanosome species* transmitted by tsetse flies in Petauke District, Eastern Province of Zambia where a trial of restricted application of insecticide trial was to be conducted (Chapter six).

5.2. Material and methods

Blood samples were collected from 958 Angoni (Zebu) traditional cattle at twelve villages in Petauke District (Figure 5.1, 5.2, 5.7 and 5.8) from 31st May 2004 to 12 June 2004 (end of rainy season and beginning of cool season). The twelve villages were purposively selected from villages that had high prevalence in the previous surveys conducted in the area (Hopkins *et al*, 1998, Machila *et al.*, 2001; Sinyangwe, L. *et al*, 2004) and accessible throughout the year. The minimum number of animals that was required to be in each village was estimated using the following equation (Cannon and Roe, 1982; Thrusfield, 2000):

$$n = \frac{t^2 P_{exp}(1 - P_{exp})}{d^2}$$

n = sample size

t = student T value (1.96 for 95% confidence level)

P_{exp}= prevalence of 0.05 (baseline screening results: chapter 5)

d = desired absolute precision or power (5%)

From the given figure above the minimum number of cattle required in each of the 12 villages;

$$n = \frac{1.96^2 \ 0.05 \ (1 - 0.05)}{0.05^2} = 72.99 \text{ cattle per treatment group}$$

This gave a figure of about 73 cattle per village. Since these animals were to be used in a longitudinal study (chapter 6) where attendance becomes poor as the study processes, it was decided to recruit between 70-80 cattle in each village. Eighty cattle were selected and sampled from animals that were presented by farmers in each village apart from Chikuse where all 78 cattle present were sampled. Eighty was also a maximum number of cattle a sampling team could sample comfortably in a day. Cattle of all ages and sex were included in the sample. A proportion of cattle from each farmer present on the day of sampling were included in the sample. To avoid selection of only sick animals or those that were preferred by the farmers, cattle from each farmer were randomly selected at the crushpen on the day of sampling.

The prevalence of the tsetse and tick-borne parasites were determined by both microscopic examination of thick and thin smears stained with Giemsa stain and PCR amplification methods (described in chapter two of this thesis). Trypanosomes

and *Theileria* parasites were diagnosed by both microscopy and PCR while *Anaplasma, Babesia* parasites were diagnosed only by microscopy. The p104 gene PCR using nested forward and reverse internal primers that generated product of 278bp was used for *Theileria parva* and the Nested Internal Transcribed Spacer PCR (ITS) for trypanosomes (Cox, *et al*, 2005)

5.3. Results

5.3.1. Tick-borne diseases

5.3.1.1. Number of cattle positive for *Theileria species* by microscopy and PCR

In order to obtain baseline data of the prevalence and distribution of the *Theileria* species in the study area, cattle were examined for the parasites by both microscopy and PCR technique (before the insecticide trial described in chapter 6 commenced). This was done 42 days before the application of insecticide started. The results for both microscopy (micro.) and PCR examinations are shown in Table 5.1 and Figures 5.1 and 5.2 below. Based on the prevalence as determined by microscopy and PCR, the 12 villages can be grouped into three groups;

Group one - These were villages that had higher prevalence (p) of *Theileria spp* by microscopy ($5\% < p \leq 20\%$) but low by PCR ($p \leq 5\%$). Villages in this group included Chipungu (micro. = 20% [95%CI: 12.7% to 30%]; PCR = 5% [95%CI: 2% to 12.2%]), Makale (micro. = 11.25% [95%CI: 6% to 20%]; PCR = 3.75% [1.3% to 10.5%]) and Mnyeche (micro. = 6.25% [95%CI: 2.7% to 13.8%]; PCR = 5% [2% to 12.2%]). The other village that was identified with this group was Chikuse that had prevalence of less than 5% (95%CI: 0.7% to 8.9%) by both PCR and microscopy examination.

Group two - The second group comprised Nyamphande, Chipembe and Nkhunta. Compared with other groups, villages in this group showed low to medium prevalence by both microscopy ($2.5\% \leq p < 10\%$) and PCR ($5\% < p \leq 15$). The prevalence at Nyamphande was 2.5% (95%CI: 0.7% to 8.7%) by microscopy and 8.75% (95%CI: 4.3% to 17%) by PCR. At Chipembe it was 8.75% (95%CI: 4.3% to 17%) by microscopy and 11.25% (95%CI: 6% to 20%) by PCR. The prevalence at Nkhunta was 7.5% (95%CI: 3.5% to 15.4%) by microscopy and by PCR was 15% (95%CI: 8.8% to 24.4%).

Group three - The villages in the last group included Kasonde, Manga, Chalokwa, Sikwenda and Kasero. These villages showed high prevalence ($p > 25\%$) of *Theileria*

spp by PCR and low to medium prevalence (5% < x <20%) by microscopy. The prevalence were at Sikwenda (PCR = 32.5% [95%CI: 23.2% to 43.4%]; micro. = 7.50% [95%CI: 3.5% to 15.4%]), Kasero (PCR = 32.5% [95%CI: 23.2% to 43.4%]; micro. = 6.25% [95%CI: 2.7% to 13.8%]), Chalokwa (PCR = 30% [95%CI: 21.1% to 40.8%]; Micro. = 16.2% [95%CI: 9.7% to 25.8%]), Manga (PCR = 27.5% [95%CI: 18.9% to 38.1%]; micro. = 8.75% [95%CI: 4.3% to 17%]) and Kasonde (PCR = 26.2% [95%CI: 17.9% to 36.8%]; micro. = 7.50% [95%CI: 3.5% to 15.4%]).

Figure 5. 1Prevalence (%) of *Theileria spp* in cattle by microscopy and PCR.

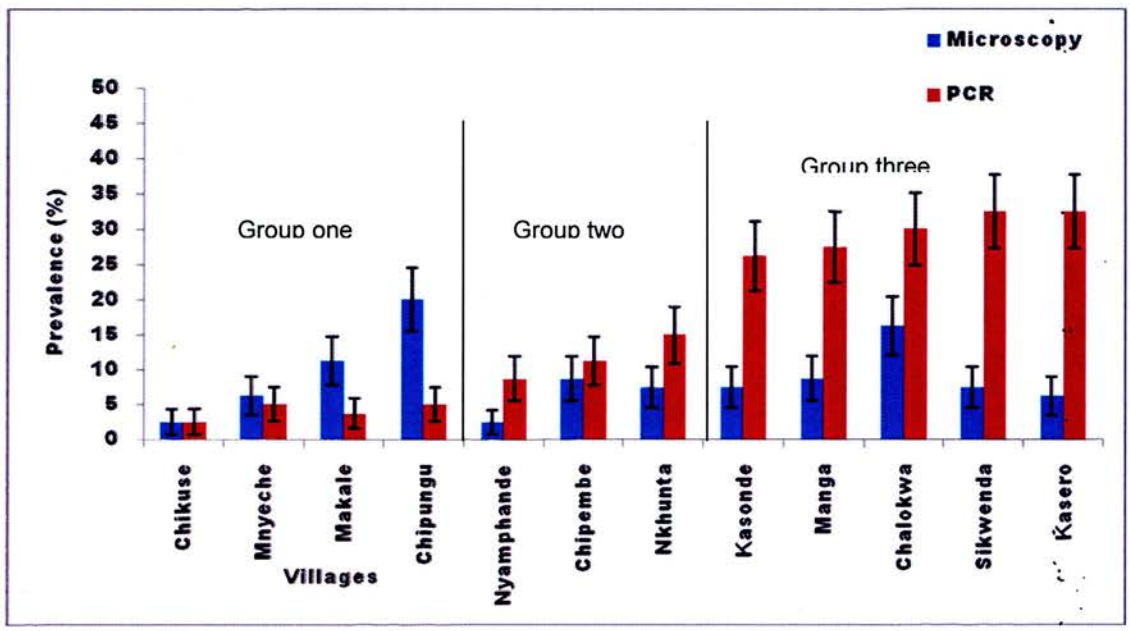
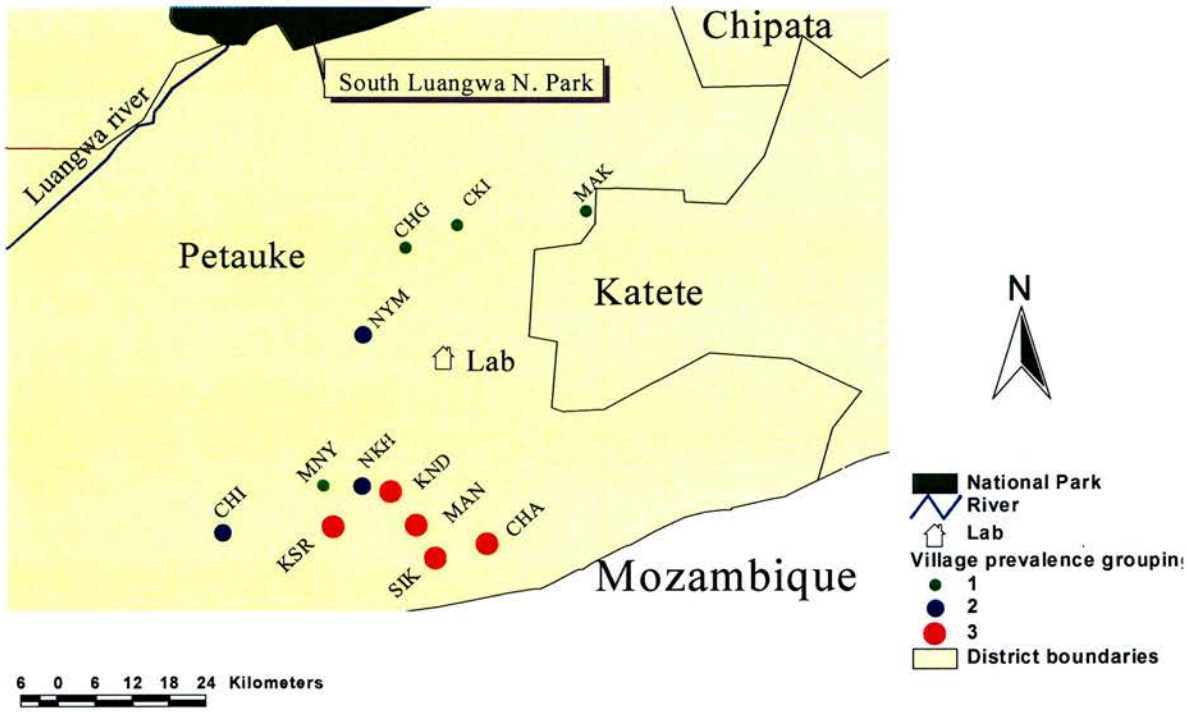


Table 5. 1Number of cattle positive for *Theileria spp* by microscopy and PCR (Baseline data)

	Village	Number of cattle	Number postive by Microscpy	Prev (%)	Number positive by PCR	Prev (%)	Positive by both methods	Total positive
1	Chikuse	78	2	2.56	2	2.56	0	4
2	Mnyeche	80	5	6.25	4	5	0	9
3	Makale	80	9	11.3	3	3.75	1	11
4	Chipungu	80	16	20	4	5	1	19
5	Nyamphande	80	2	2.5	7	8.75	1	8
6	Chipembe	80	7	8.75	9	11.2	5	11
7	Nkhunta	80	6	7.5	12	15	1	17
9	Kasonde	80	6	7.5	21	26.2	5	22
7	Manga	80	7	8.75	22	27.5	2	27
6	Chalokwa	80	13	16.2	24	30	9	28
10	Sikwenda	80	6	7.5	26	32.5	4	28
11	Kasero	80	5	6.25	26	32.5	2	29
	Grand total	**958**	**84**	**8.77**	**160**	**16.7**	**31**	**213**

Figure 5. 2 *Theileria* parasites prevalence at the twelve villages in Petauke District.



Group 1 (•): Microscopy (5% < x ≤ 20%) and PCR (x ≤ 5%)

Group 2 (•): Microscope (2.5% ≤ x <10%) and PCR (5% < x ≤ 15)

Group 3 (•): Microscopy (5% < x <20%) and PCR (x > 25)

Lab = Laboratory at Petauke District Veterinary office.

Abbreviations

CHA = Chalokwa	SIK = Sikwenda
CHI = Chipembe	MAK = Makale
CHG = Chipungu	MAN = Manga
CKI = Chikuse	MNY = Manyane
KSR = Kasero	NHK = Nkhunta
KND = Kasonde	NYM = Nyamphande

5.3.1.2. Test of agreement between microscopy and PCR results

During baseline data collection a total of 958 cattle were examined for *Theileria parasites* (Table 5.2). Out of this number 31 cattle (3.24%) were found to be positive for *Theileria species* by both PCR and microscopy, 129 (13.5%) were positive by PCR but negative by microscopy. Fifty three (5.53%) cattle were positive for *Theileria spp* by microscopy but negative by PCR. The number that was found negative by both methods was 745 (77.77%). The total number of cattle that were found positive by either microscopy or PCR was 213 (22.23%).

Based on these baseline data results, PCR was able to detect twice the number of *Theileria* parasites that was detected by microscopy. In total microscopy detected 84 (8.77%) positives while PCR detected 160 (16.70%). This represented a ratio (microscopy:PCR) of 1:2. The difference in prevalence between microscopy and PCR was highly significant (χ^2 = 27.01, df = 1, p-value < 0.001). Conversely, the kappa value of agreement was fairly low, 0.157 (Table 5.2).

Table 5. 2 Test of agreement between microscopy and PCR results.

		Microscopy		
		Positive	**Negative**	**Total**
PCR	**Positive**	31	129	160
	Negative	53	745	798
	Total	84	874	**958**

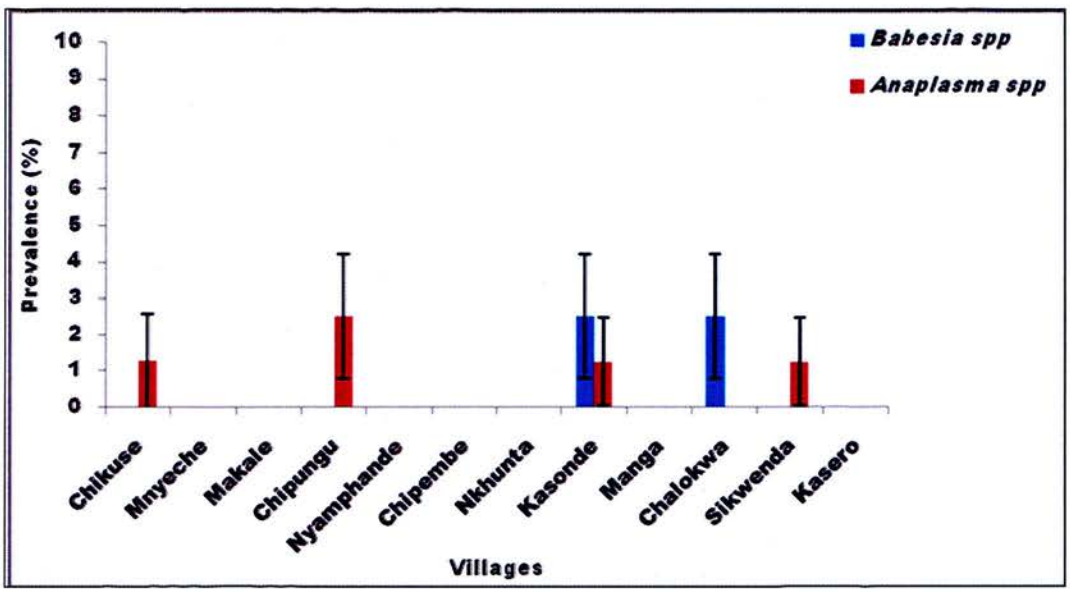
Kappa value = 0.157

5.3.1.3. Prevalence of other tick-borne parasites in Petauke District.

The prevalence of *Anaplasma spp* and *Babesia spp* in Petauke District as diagnosed by microscopy is as shown in Figure 5.3. Very few animals were found to be infected with *Anaplasma spp* and *Babesia spp*. *Anaplasma spp* were only diagnosed at Chikuse (1/78[1.28%, 95%CI= 0.2% to 6.9%], Chipungu (2/80[2.5%, 95%CI: 0.7%

to 8.7%]), Kasonde (1/80[1.25%, 95%CI: 0.2% to 6.7%]) and Sikwenda (1/80[1.25%, 95%CI: 0.2% to 6.7%]). *Babesia spp* were diagnosed at Kasonde (2/80(2.50%, 95%CI: 0.7% to 8.7%)) and Chalokwa (2/80(2.50%, 95%CI: 0.7% to 8.7%)).

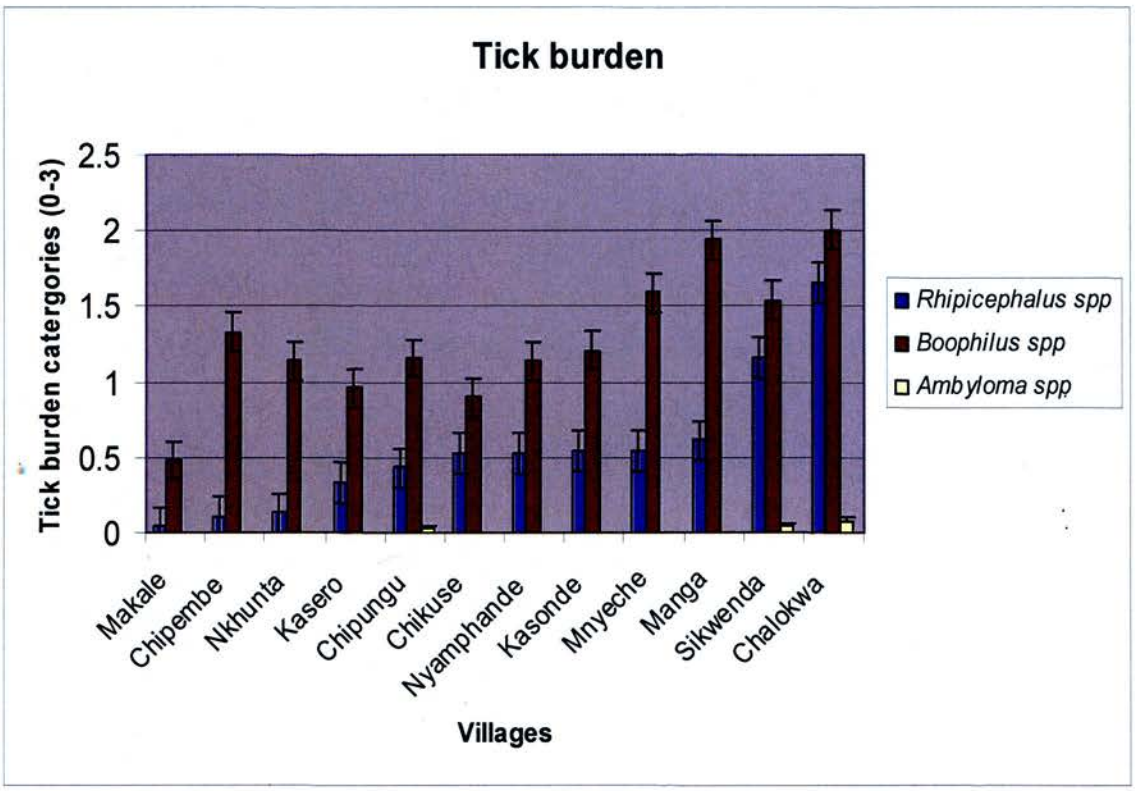
Figure 5. 3 Prevalence (%) of *Anaplasma spp* and *Babesia spp* (Baseline data) microscope.



5.3.1.4. Tick burden (baseline data)

During baseline data collection, there were very few *Amblyomma spp* seen on the animals. A few were only seen at Chipungu, Sikwenda and Chalokwa. Many *Boophilus spp* were seen at all the villages. *Rhipicephalus spp* were also seen at all the villages but the number was less than that of *Boophilus spp* (Figure 5.4).

Figure 5. 4 Average number of ticks per animal in the 12 villages of Petauke District (Baseline data)



Zero (0) represented no tick found on the body, 1 (low or mild) when number of ticks was between 1 and 10, 2 (medium or moderate) when number of ticks were between 11 and 50 and 3 (high or severe) when number of ticks was over 50.

5.3.1.5. Mean Haemoglobin values (g/dl) for Theileria positive and negative cattle

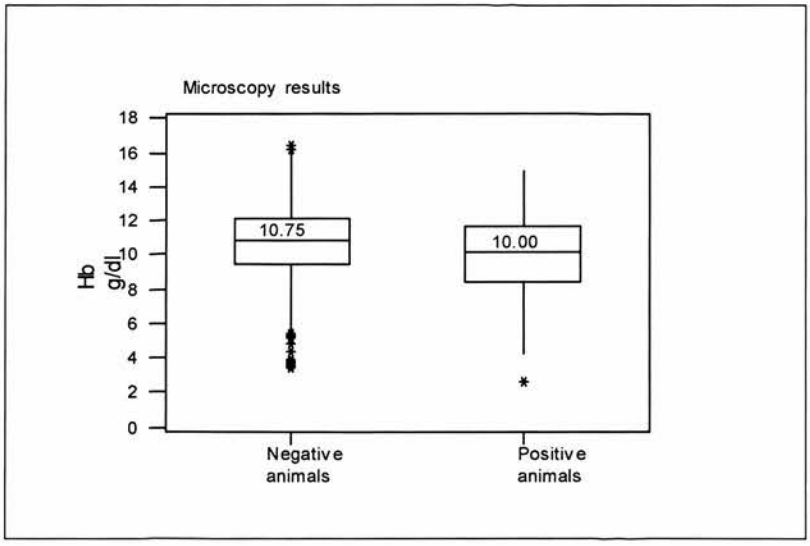
The mean haemoglobin value of the animals that were positive for *Theileria* parasites by microscopic examination was 10.0 g/dl. The mean value for those animals that were negative for the *Theileria* parasites by microscopy was 10.8 g/dl (Table 5.3 and Figure 5.5). The difference was statistically significant (t-test; p= 0.008). Although the mean haemoglobin value (Table 5.4 and Figure 5.6) of the animals positive (10.5 g/dl) by PCR was lower than that of the negative animals (10.7 g/dl), the difference was not statistically significant (t-test; p = 0.216).

Table 5. 3 Mean haemoglobin (g/dl) values for animals positive and negative for *Theileria* parasites by microscopy.

	Mean	Max	Min	Stdev	Median	SE Mean	n
Negative animals	10.8	16.4	3.50	2.12	10.9	0.37	874
Positive animals	10.0	14.9	2.60	2.45	10.1	1.10	84

Stdev = Standard deviation, SE Mean= Standard error of the mean

Figure 5. 5 Mean Haemoglobin (g/dl) values for animals positive and negative for *Theileria* parasites by microscopy.



75th centile (upper quartile) = 75% of the observations lie below and 25% above this value.

Median (50th centile or quantile) = value below which half, and above which half, of the observations lie.

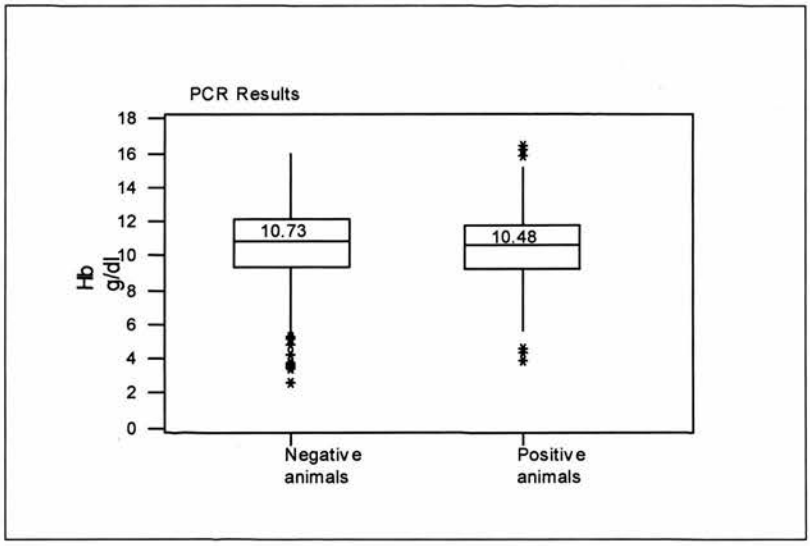
25th centile (lower quartile) = 75% of the observations lie above it and 25% below this value. (M. Thrusfield, 2000)

***** = Outliers**

Table 5. 4 Mean Hb values for animals positive and negative for trypanosomiasis by PCR.

	Mean	Max	Min	Stdev	Median	SE Mean	n
Negative animals	10.7	15.9	2.6	2.13	10.9	0.39	798
Positive animals	10.5	16.4	3.9	2.30	10.6	0.84	160

Figure 5. 6 Mean Hb values for animals positive for *Theileria* parasites by PCR.



5.3.2. Trypanosomiasis

5.3.2.1. Number of cattle positive for Trypanosomes by microscopic examination and PCR

The prevalence of trypanosomes infection detected by either microscopy or by PCR in each of the 12 villages are shown in Table 5.5 and Figures 5.7 and 5.8. Trypanosomiasis cases were more prevalent in villages in the northern part of Petauke District (Table 5.5 and Figures 5.7 and 5.8). High prevalence of trypanosome infection was recorded at Makale by both microscopic examination 18/80 (22.5%, 95%CI: 14.7% to 32.8%) and PCR 14/80(17.5%, 95%CI: 10.7% to

27.3%), Chipungu 12/80 (15%, 95%CI: 8.8% to 24.4%) by PCR, 2/80 (2.5%, 95%CI: 0.7% to 8.7%) by microscopy and at Nyamphande 11/80 (13.75%, 95%CI: 7.9% to 23%) by PCR and by microscopic examination was 5/80 (6.25%, 95%CI: 2.7% to 13.8%). Chalokwa was the only village on the southern part of the district that recorded high prevalence 10/80 (12.5%, 95%CI: 6.9% to 21.5%) by PCR (Table 5.5 and Figures 5.3 and 5.8).

Table 5. 5 Trypanosomiasis prevalence (%)

		PCR		Thick & thin smear	
Sample number	**Village**	**Number of infected animals**	**Prev (%)**	**Number of infected animals**	**Prev (%)**
80	Makale	14	17.5	18	22.5
80	Chipungu	12	15	2	2.5
80	Nyamphande	11	13.75	5	6.25
80	Chalokwa	10	12.5	1	1.25
78	Chikuse	3	3.85	5	6.41
80	Chipembe	2	2.5	0	0
80	Kasero	1	1.25	0	0
80	Kasonde	1	1.25	1	1.25
80	Mnyeche	1	1.25	0	0
80	Sikwenda	0	0	0	0
80	Manga	0	0	0	0
80	Nkhunta	0	0	0	0
958		**55**	**5.74**	**32**	**3.26**

Figure 5. 7 Trypanosomiasis prevalence (%) at the 12 villages of Petauke District.

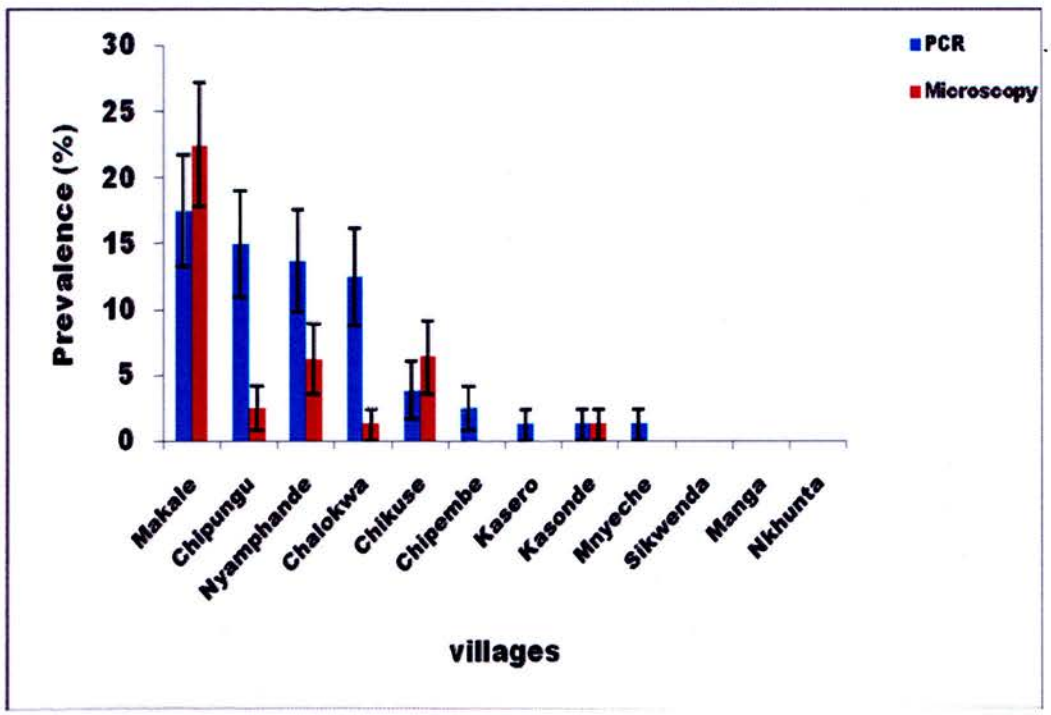
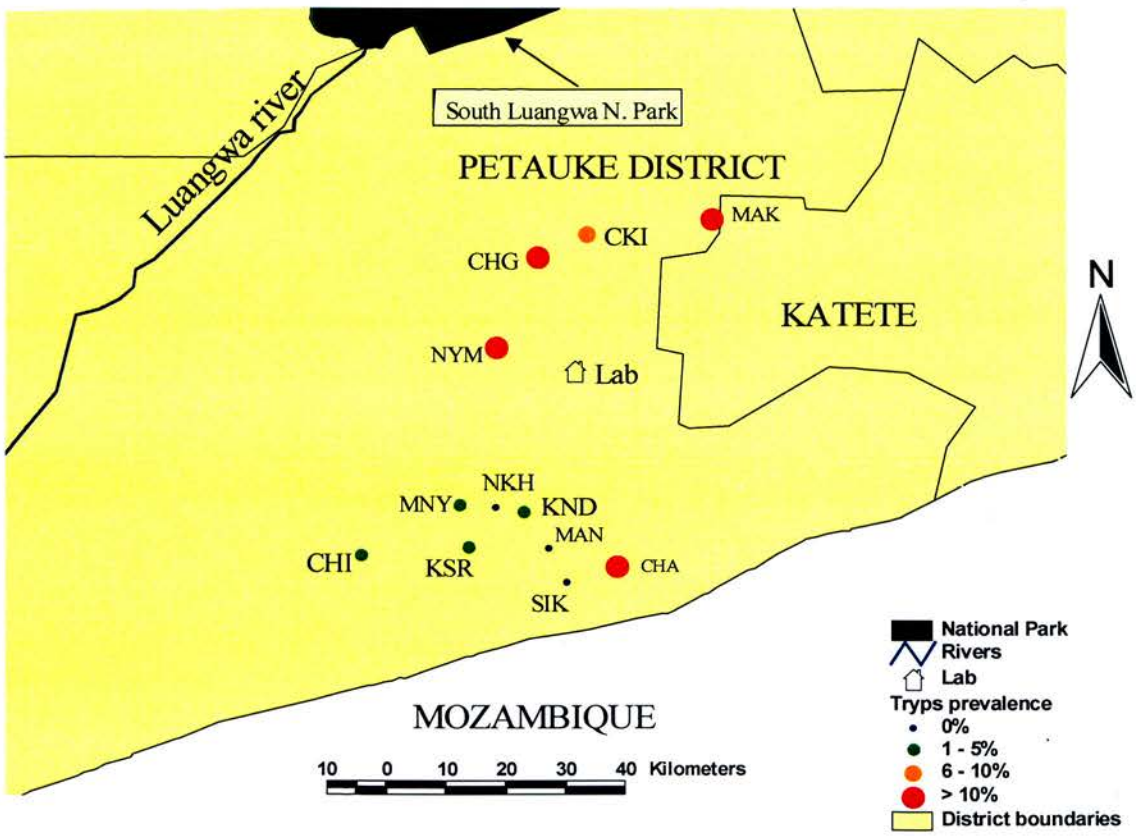


Figure 5. 8 Trypanosomiasis prevalence at the 12 villages in Petauke District.



Group one(•): prevalence = 0% **Group two (•): Prevalence = 1% - 5%**

Group three (•):Prevalence = 6% - 10%, **Group four (•): Prevalence = >10%**

Abbreviations

CHA = Chalokwa	SIK = Sikwenda
CHI = Chipembe	MAK = Makale
CHG = Chipungu	MAN = Manga
CKI = Chikuse	MNY = Manyane
KSR = Kasero	NHK = Nkhunta
KND = Kasonde	NYM = Nyamphande

Lab = Laboratory at Petauke District Veterinary office

5.3.2.2. Test of agreement between microscopy and PCR results

Out of a total number of 958 cattle that were examined for trypanosomes, 11 cattle (1.15%) were found to be positive for the parasites by both PCR amplification and microscopic examination of the thick and thin smears stained with Giemsa stain (table 5.6). Forty four (4.59%) were positive by PCR but negative by microscopy. Twenty one (2.19%) cattle were positive for the parasites by microscopy but negative by PCR. The number that was found negative by both methods was 882 (99.07%).

The total number of animals that were positive by either microscopy or PCR were 76 (7.93%).

These results revealed that ITS-PCR was able to detect twice the number of infections that were detected by microscopy. The total number of cattle found positive for the parasites by microscopy were 32 (3.34%) while PCR detected 55 (5.74%). This represented a Ratio (Microscopy/PCR) of 1:2. The difference in prevalence between microscopy and PCR was highly significant ($\chi^2 = 6.370$, df = 1, *p*-value = 0.012). The kappa value for concordance was 0.22 (Table 5.6).

Table 5. 6 Microscopy and PCR results for cattle (n = 958).

		Microscopy		
		Positive	**Negative**	**Total**
PCR	**Positive**	11	44	55
	Negative	21	882	798
	Total	32	874	**958**

Kappa value = 0.22

5.3.2.3. Trypanosome species in cattle from Petauke District

The percentages of all trypanosome infections are shown in Table 5.5. The common species (Table 5.5) of trypanosomes was *T. congolense* (65.6% by microscopy and 58.2% by PCR) followed by *T. vivax* (18.8 by microscopy and 38.2% by PCR). The

remaining was *T. brucei* (15.6% and 3.64% by microscopy and PCR respectively). Sixteen cattle were positive for *T. theileri* (non-pathogenic trypanosomes) by only PCR amplification. Microscopic examination of the thick and thin smear examination did not detect any non-pathogenic trypanosomes (Table 5.7).

Table 5. 7 Species of trypanosomes (PCR and microscopy). n = 958

Thick and Thin smears			**PCR**		
Species	Total Pos	%	Species	Total Pos	%
Tc	21	65.62	*Tc*	32	58.18
Tv	6	18.75	*Tv*	21	38.18
Tb	5	15.62	*Tb*	2	3.64
Total	**32**	**100**		**55**	**100**

5.3.2.4. Mean Haemoglobin level (g/dl) for the positive and negative cattle

The mean haemoglobin value of animals that tested positive for trypanosomes by microscopy (9.2 g/dl) was lower than that of the negative animals (10.7g/dl). The same was observed for the animals that were positive by PCR; the mean for the PCR positive animals was 9.25 g/dl while it was 10.77 g/dl for the negative ones (Tables 5.8 and 5.9 and Figure 5.9a and 5.9b). This was statistically significant in both cases (t-test; $p < 0.001$)

Table 5. 8 Mean haemoglobin value for animals positive and negative for trypanosomiasis by microscopy

	Mean	**Max**	**Min**	**Stdev**	**Median**	**SE Mean**	**Total**
Negative animals	10.7	16.4	2.60	2.15	10.9	0.36	**926**
Positive animals	9.18	13.4	5.70	1.98	8.90	1.57	**32**
							958

Figure 5. 9a Mean haemoglobin values for animals positive and negative for trypanosomes by microscopy.

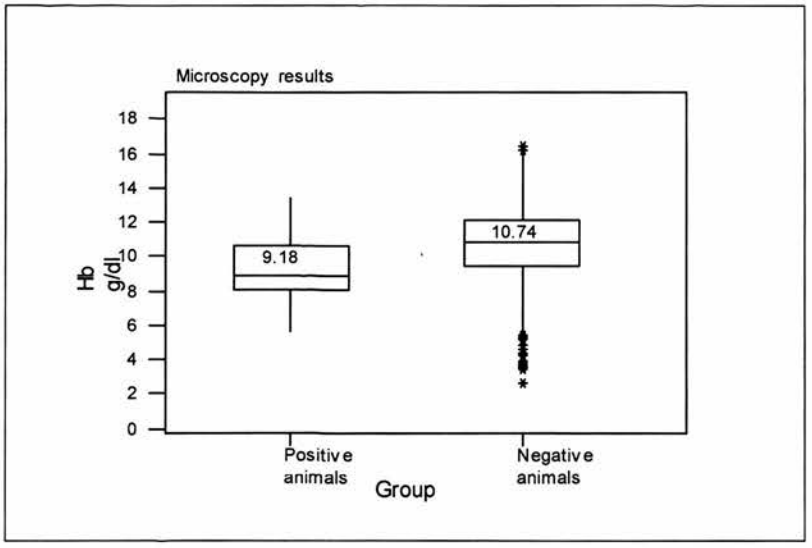
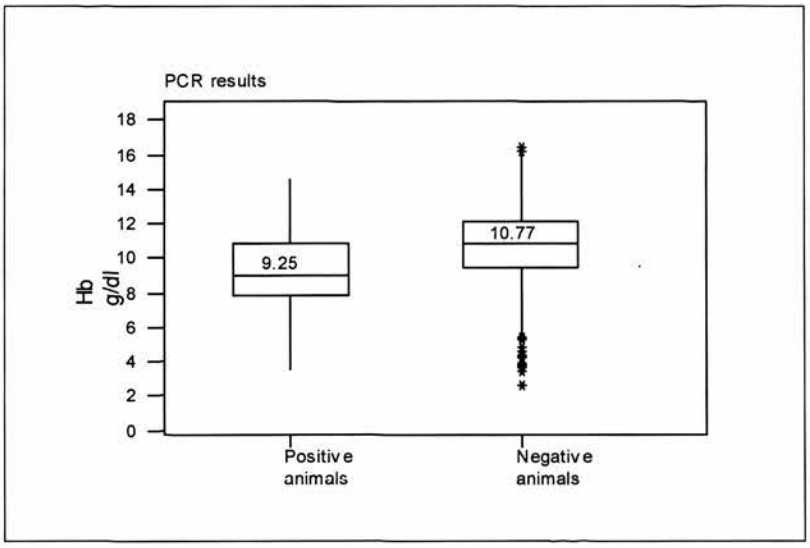


Table 5. 9 Mean haemoglobin values for animals positive and negative for trypanosomiasis by PCR

	Mean	Max	Min	Stdev	Median	SE Mean	n
Negative animals	10.8	16.4	2.60	2.12	10.9	0.07	**903**
Positive animals	9.25	14.5	3.60	2.27	9.00	0.31	**55**
							958

Figure 5.9b Haemoglobin values for animals positive and negative for trypanosomes by PCR.



5.3.3. Mean haemoglobin (g/dl) values for the individual villages

The mean haemoglobin values (Table 5.10 and Figure 5.10) of animals in the 12 villages in Petauke District was highest at Chikuse (mean =11.54 g/dl; range = 2.6g/dl – 15.9g/dl) and the second highest was at Kasero (mean = 11.30g/dl; range 5.7-15.3g/dl). Makale had the lowest mean haemoglobin value (Mean = 9.13g/dl; range = 3.6g/dl – 14.6g/dl) and the proportion of the number of anaemic animals was also more at this village (26/80 = 32.5%). See Table 5.5 and Figure 5.7.

Figure 5.10 Mean haemoglobin (g/dl) values per village

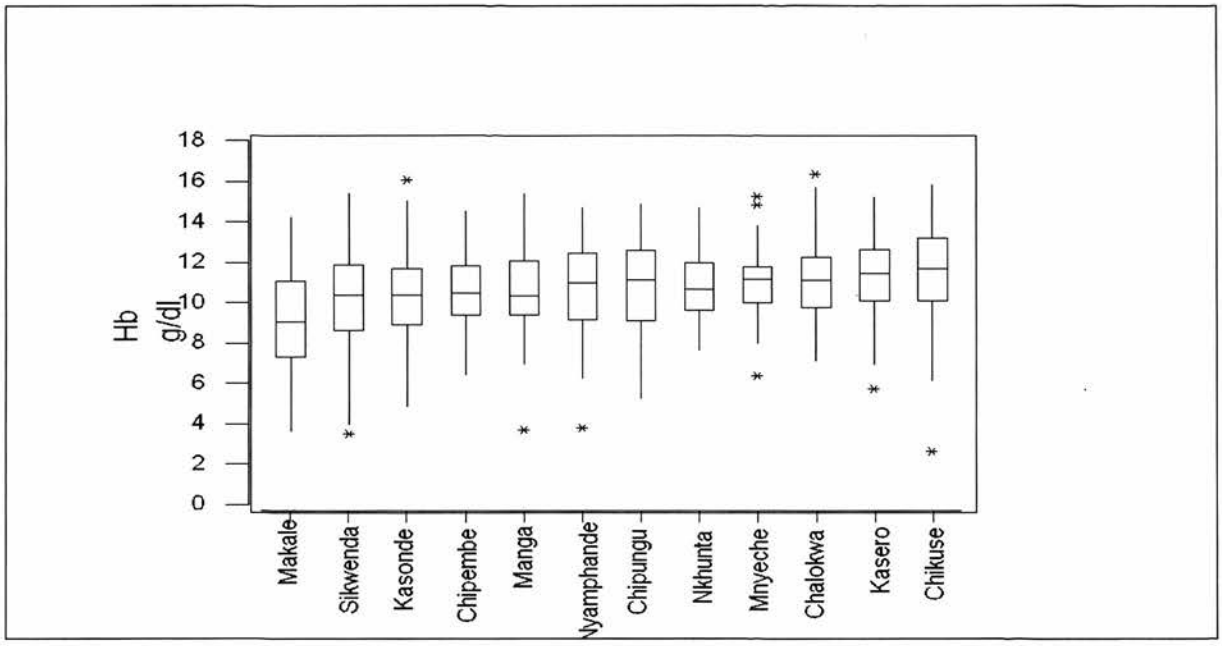


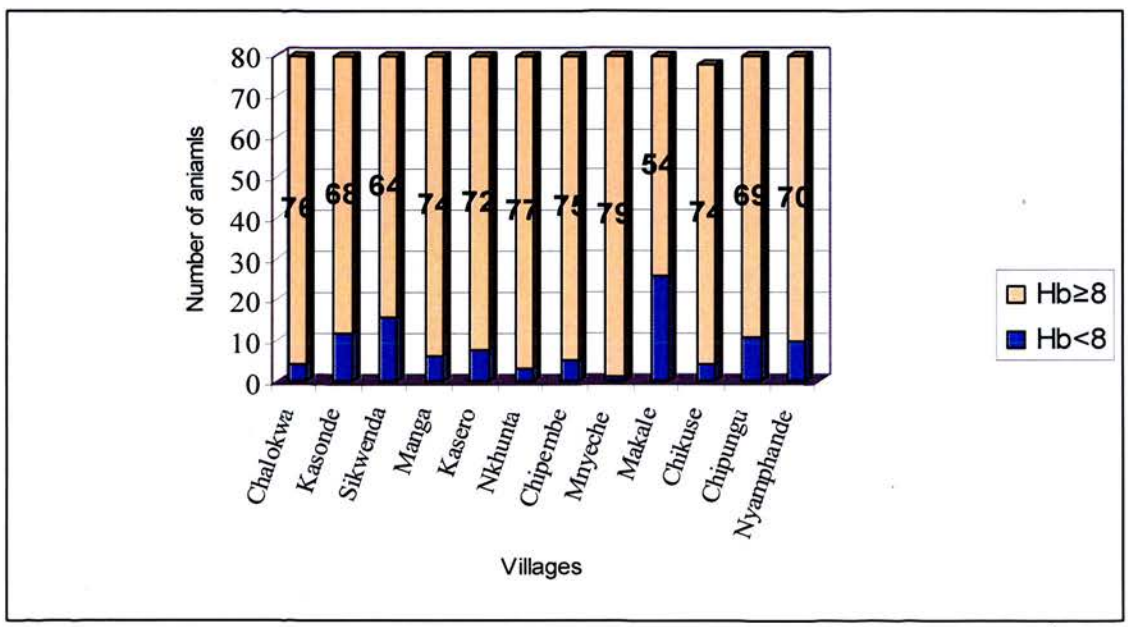
Table 5. 10 Mean haemoglobin (g/dl) values per village and number of animals with Hb above 8g/dl and Hb below 8g/dl.

Village	Mean	Stdev	Min	Max	Hb<8	Hb≥8	n
Mnyeche	11.10	1.572	6.4	15.3	1	79	80
Nkhunta	10.88	1.60	7.6	14.8	3	77	80
Chalokwa	11.14	1.96	7.1	16.4	4	76	80
Chipembe	10.58	1.70	6.4	14.6	5	75	80
Chikuse	11.54	2.27	2.6	15.9	4	74	78
Manga	10.61	1.89	3.7	15.5	6	74	80
Kasero	11.30	2.05	5.7	15.3	8	72	80
Nyamphande	10.74	2.21	3.8	14.8	10	70	80
Chipungu	10.81	2.38	5.2	15.0	11	69	80
Kasonde	10.33	2.14	4.8	16	12	68	80
Sikwenda	10.09	2.62	3.5	15	16	64	80
Makale	9.13	2.32	3.6	14.3	26	54	80

5.3.4. Proportion of animals that were anaemic (Hb < 8 g/dl)

The village (Table 5.10 and Figure 5.11) that had the highest number of animals that were considered to be anaemic (Hb< 8g/dl) was Makale (26/80 = 32.5 %) and the second was Sikwenda (16/80 = 20%). The village with least number of animals that were anaemic was Mnyeche (1/80 = 1.25%) and the second with the least anaemic animals was Nkhunta (3/80= 3.75%). The difference in the proportion of number of animals that were considered to be anaemic at Makale and Mnyeche was statistically significant between these two villages (χ^2= 25.6642, 95% CI: from -0.4304833 to -0.1945167, d.f. = 1, p-value < 0.001).

Figure 5.11 Number of animals with Hb < 8 and Hb ≥ 8g/dl in the 12 villages of Petauke District



n =80 except at Chikuse where n = 78

5.3.5. Mean haemoglobin values (g/dl) for the animals that were positive for both Trypanosomes and theileria parasites

The mean haemoglobin value for the animals that were negative for either trypanosomes or theileria parasites (10.9g/dl) was higher than those for the animals that were positive for either trypanosmes (9.33 g/dl) or theileria (10.5g/dl) or both parasites (9.17g/dl). See table 5.11 and Figure 5.12.

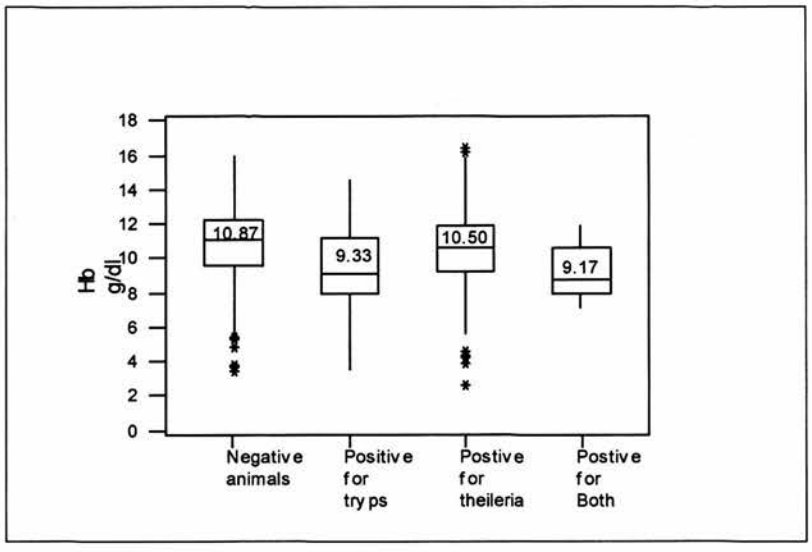
The difference between the mean haemoglobin value of negative animals (10.9g/dl) and animals that were positive for both trypanosome and *Theileria* parasites (9.17g/dl) was statistically highly significant (95%CI: from 0.665 to 2.73, t = 3.23, $p < 0.001$, d.f. = 707).

The difference between the mean haemoglobin value of negative animals and those that were positive for trypanosomes (9.33 g/dl) was also highly significant (95%CI: from 0.955 to 2.12, t = 5.18, $p < 0.001$, d.f. = 743). The difference between the mean haemoglobin value of negative animals and animals that were positive for theileria parasites (10.50g/dl) was approaching significance (95%CI: from 0.330 to 0.702, t = 2.16, $p = 0.031$, d.f. = 890)

Table 5. 11 Mean haemoglobin values (g/dl) for the animals that were positive for both Trypanosomes and theileria parasites

	Mean	Max	Min	Stdev	Median	SE Mean	**n**
Negative animals	10.9	15.9	3.50	2.02	11.1	0.08	**694**
Positive for trypanosomes	9.33	14.5	3.60	2.40	9.10	0.34	**51**
Positive for theileria	10.5	16.4	2.60	2.41	10.6	0.17	**198**
Positive for both	9.17	11.9	7.10	1.53	8.80	0.39	**15**
							958

Figure 5.12 Mean haemoglobin values (g/dl) for the animals that were positive for both Trypanosomes and theileria parasites (baseline data)



5.4. Discussion

5.4.1. Tick-borne diseases

The results showed that *Theileria parva* parasites as detected by PCR were more prevalent in the villages that lie in the southern part of Petauke District. The district on the southern is a plateau and gently slopes into the valley on the northern part. The prevalence was also high in the eastern part of the district and low in western part (Figure 5.2). This is in agreement with the findings of Berkvens *et al* (1998) on the spread of their vector *Rhipicephalus appendiculatus* in the same area, who considered that the spread of the disease from the eastern to western and south to northern has been mainly through movement of cattle mostly working oxen. These oxen transport farm produce and go to work in neighbouring or distant villages. When grazing cattle also spread the infection as they can sometimes travel a distance of 15km or more when looking for good pasture (Billiouw, *et al*, 1999; Billiouw, *et al*, 2002). *Theileria spp* as revealed by microscopic examination in general followed the same pattern, apart from high prevalences that were observed at Makale and Chipungu. This suggested that microscopic examination detected other species of *Theileria* species that were not amplifed by PCR. The PCR amplification (Iams, *et al*, 1990, Skilton, *et al*, 2002) used primers that were specific for the *Theileria parva* $_{P}$104 gene and could not therefore detect any other parasites of *Theileria* species circulating in the area. Apart from *T. parva*, other species like *T. mutans*, *T. velifera* and *T. taurotragi* have been diagnosed in Zambia (Makala, *et al*, 2003). *Theileria velifera* and *T. mutans* are transmitted by *Amblyomma spp* and a few ticks of this species were seen at Chipungu, Sikwenda and Chalokwa during the baseline survey.

The mean haemoglobin value of animals that were positive for *Theileria* parasites was lower than that of animals that were negative for the parasites. The difference was more with those animals that were positive by microscopic examination than those positive by PCR amplification. The study showed that anaemia in animals that were positive for *Theileria* parasites by PCR was not significant were as in the animals that were positive by microscopy it was. It seems PCR was detecting sub-clinical parasitaemic cases where as microscopy detected clinical cases. It had been observed that anaemia is not a major diagnostic sign since there is minimal division

of the parasites in RBC, and thus no massive destruction of them (Young *et al*, 1988; The Merck Veterinary Manual, 1991).

There were very few animals that were found with anaplasma parasites during the baseline survey. One possible explanation of this observation is that the *Amblyomma species* that transmit these parasites were also in low numbers during this time (Figure 5.4). The other reason could be due to heavy use of tetracycline antibiotic in the area (Personnal observation) before commencement of the study. These drugs have a great impact on anaplasma parasites and could have masked the disease. It could also be that the local cattle are tolerant compared to non-resistant exotic animals (Young *et al,* 1987b; Young *et al*, 1988; Minjauw and Mcleod 2003).

Despite the abundance of the vector (*Boophilus spp*) that transmitt *Babesia* parasites (Figure 5.4), only a small number of animals were found positive for *Babesia* parasites. The low prevalence of *Babesia* parasites in animals could be due to the fact that many farmers in this area use trypanocidal drugs (diminezene diaceturate) to treat the animals for suspected causes of trypanosomiasis. These drugs are known to be effective against the *Babesia* parasites. The availability of the antibiotics and trypanocidal drugs to the farmers at the District Veterinary Office has made it easy for them to buy them at any time. The purchasing of the drugs scheme that was introduced by the Assistance of Veterinary Service to Zambia (ASVEZA East) a Belgian project in the area from the late 1980s to early 2000. This scheme continued even after the end of the project (Personnal communication, District Veterinary Officer, Petauke), and is now conducted by local veterinary officers at individual level (personal observation). The other reason why there were few cases of *Babesia* parasites could also be like in the case of Anaplasma were the disease tend to be more prevalent in non-resistant exotic animals (Minjauw and Mcleod 2003).

5.4.2. Trypanosomiasis

Northerly villages generally had higher prevalence as revealed by microscopic examination of thick and thin blood smears (Figure 5.8). The same picture was maintained by PCR results with the exception of Chalokwa, which is on the southern part that showed low prevalence by microscopy examination (1.25%) but high

prevalence by PCR amplification (12.5%). The northerly villages are close to the South Luangwa National Park. High prevalence in northerly villages suggests that the National Park is probably the source of infection for these villages. The one exception, Chalokwa village, may have had an alternative source of infection. The findings of this study are in agreement with the results of the previous works that were conducted in the area (Machila *et al*, 2001; Sinyangwe *et al*, 2004).

The results were consistent with the National Park being the source of tsetse flies transmitting the infection. The National Park and its surrounding Game Management Areas harbours tsetse flies and abundant wildlife that are reservoirs of trypanosomes (Kinghorn *et al*, 1913; Buyst, 1977; Van den Bossche, 2001). At Chalokwa the source of infection is mostly likely to be Mozambique, where tsetse control has never been conducted because of the civil unrest.

The data show the association between trypanosomiasis and anaemia in cattle. In this study the mean haemoglobin (Hb) value of the animals that were positive for trypanosomes by PCR was 9.25 g/dl and was lower than that of the negative animals whose mean haemoglobin value was 10.77 g/dl. This was also observed by the cattle that were found positive by microscopic examination of the thick and thin smears stained with Giemsa stain. In this case positive animals had mean haemoglobin value of 9.18g/dl and the mean value for negative animals was 10.74g/dl.

5.4.3. Mean Haemoglobin values (g/dl) in cattle in the 12 villages of Petauke district

The northern most village, Makale had the lowest mean haemoglobin value (Mean = 9.13g/dl; range = 3.6g/dl – 14.6g/dl) and the proportion of the number of anaemic animals was also more at this village (26/80 = 32.5%). This may be attributed to the high prevalence of trypanosomaisis (p > 15%) as shown by both PCR and microscopic examination of the thick and thin blood smear (Table 5.5 and Figure 5.7).

The high mean haemoglobin value (g/dl) at Chikuse may be attributed to the fact that the prevalence of trypanosomiasis by both PCR and microscopy was the lowest among the four villages on the northern part of the Study area. This is despite being in the northern part of the district that is near the source of tsetse flies. The prevalence of *Theileria* parasites was also the lowest ($p < 5\%$) among the 12 villages by both PCR and microscopy (Table 5.1 and Figure 5.1).

5.4.4. Haemoglobin values (g/dl) in cattle that were infected with both trypanosomes and theileria pararsites

The animals that were positive for both trypanosomes and *Theileria* parasites by either PCR or Microscopy had lower mean haemoglobin value (9.17g/dl) than the animals that were only positive for trypanosomes (9.33g/dl) or only positive for *Theileria* parasites (10.50g/dl). (Table 5.11 and Figure 5.12). Co-infection with other diseases has been observed to influence the severity of anaemia in animals that are infected with trypanosomes (Murray *et al*, 1982).

6. CHAPTER SIX

6 STUDY OF THE EFFECT OF THE RESTRICTED APPLICATION OF DELTAMETHRIN ON CATTLE TO CONTROL TSETSE- AND TICK-BORNE DISEASES IN ZAMBIA

6.1. Introduction

Considerable effort has been made to control tick and tsetse-borne diseases in Zambia by the government and different donor agencies. In the Eastern Province trypanosomiasis has mainly been controlled by odour-baited insecticide impregnated targets (Vale *et al.*, 1986, Vale *et al.*, 1988; Van den Bossche P, 1997) and trypanocidal drugs (Delespaux, 2000, Van den Bossche P, 2000). Application of synthetic pyrethroid on cattle in form of pour-ons has been used on trial bases to control tsetse flies (Van den Bossche *et al.,* 2004). East Coast Fever (ECF) is routinely controlled by the method of immunization and treatment (Billiouw *et al.* 1999). These methods have their own limitations in that excess use of drugs promote drug resistance and too much use of insecticide either on cattle or targets could lead to environmental damage, acaricide resistance in tick population and increase in the number of tick-borne diseases (Eisler, *et al*, 2003). Integrated control of tsetse and tick-borne diseases in the form of restricted application of pyrethroid on cattle could give an answer to some of these problems. Restricted application of insecticide to cattle is based on the observations that tsetse feed mainly on predilection sites, namely the legs and belly of cattle (Vale et al, 1999; Torr *et al*, 2007). This reduces the requirement for insecticide and may therefore have both cost benefits and environmental benefits. Moreover, reducing the amount of synthetic pyrethroid applied to cattle and restricting the area of its application will lessen its impact on tick populations. This will help alleviate concerns that widespread use of synthetic pyrethroids to control tsetse may interfere with development of endemic stability to tick-borne diseases (Van den Bossche and Mudenge, 1999; Van den Bossche et al., 2002). Finally, reducing the amount of spray may delay the development of resistance to this class of compound in ticks (Torr *et al*, 2007)

Against this background, a trial to study the effect of the restricted application of insecticide deltamethrin on cattle to control tsetse- and tick-borne diseases was conducted in Petauke and Nyimba Districts, Eastern Province, Zambia. Eastern province was chosen for this work because trypanosomiasis has been a serious problem in this part of Zambia (Machila *et al.*, 2001; Sinyangwe, L., 2004).

Theileriosis is also a disease of great importance in the province (Berkvens, *et al*, 1998).

6.2. Objective of the chapter

The main objective of this chapter was to evaluate the effect of the restricted application of synthetic pyrethroid (deltamethrin) on cattle to control tsetse- and tick-borne diseases in Zambia. The method was also compared with other methods of controlling these diseases. The other methods were: the use of prophylactic trypanocidal drug (isometamidium chloride) and insecticide application on cattle in form of pour-ons.

The main aim of this chapter was to determine the monthly incidence of tsetse and tick-borne parasites in cattle in the 12 villages that were under different treatment regimes in Petauke and Nyimba Districts, Eastern Province, Zambia. Other aims were to determine the effect of these treatments on the haemoglobin values and body condition of cattle.

It is hypothesised that treating cattle with synthetic pyrethroid (deltamethrin) applied only to ears, bellies and limbs will result in lowering the prevalence of trypanosomiasis because tsetse mainly feed on the animals from these sites. This will also result in reduction of tick-borne diseases because deltamethrin is also used to control ticks.

6.3. Material and methods

6.3.1. Study area

The study area comprised 12 villages that were situated in Petauke and Nyimba Districts, Eastern province of Zambia. The area lies between latitudes 13° 58'S and 14° 33'S and longitudes 30° 45'E and 31° 35'E (Chapter 5; Section 5.3.1. and Figures 5.2 and 5.8).

6.3.2. Sample size

Eighty (80) cattle were recruited at each of the villages for the study apart from one village where there were only 78 because this was the number that was presented by the farmers. The minimum number of animals that was required to be in each village

was estimated using the following equation (Cannon and Roe, 1982; Thrusfield, 2000):

$$n = \frac{t^2 P_{exp}(1 - P_{exp})}{d^2}$$

n = sample size

t = student T value (1.96 for 95% confidence level)

P_{exp}= prevalence of 0.05 (baseline screening results: chapter 5)

d = desired absolute precision or power (5%)

From the given figure above the minimum number of cattle required in each of the 12 villages;

$$n = \frac{1.96^2 \ 0.05 \ (1 - 0.05)}{0.05^2} = 72.99 \text{ cattle per treatment group}$$

This gave a figure of about 73 cattle per village. An allowance of 10% was added to this figure to accommodate drop-outs that is common in longitudinal studies. In total 80 cattle were recruited at each village (see chapter 5).

6.3.3. Allocation of cattle to treatment groups

Forty-two days (day -42) prior to first intervention (day 0), cattle were ear tagged, clinically examined and treated with 7 mg/kg of diminazene aceturate (Veriben®) by intramuscular route. The dosage was repeated after 14 days (day –14) prior to first intervention. Two weeks (day 0) after the second dosage of diminazene aceturate (Veriben™, CEVA SANTE ANIMALE), animals were assigned to four different treatment groups as follows:

Group 1. Three villages were assigned to restricted application of insecticide i.e. 1:1000 aqueous solution of deltamethrin 5% m/v (Decatix®, Coopers). Insecticide was sprayed only to the legs, belly and ears of the cattle using knapsack sprayers. Ears were sprayed because of the brown ear tick (*Rhipicephalus appendiculatus*). The hair coat was saturated to the point of run off. This was done once every month for the duration of the study;

Group 2. Three villages were assigned to the pour-on treatment at 0.1 mg/kg of deltamethrin 1% w/v (Spot-on®). The pour-on was applied on the dorsal part of the animals from the head to the tail once a month;

Group 3. Animals at the other three villages were treated with Isometamidium chloride hydrochloride (Veridium^{TM}, CEVA SANTE ANIMALE) at 1 mg/kg body weight (0.05ml/kg) of 2% solution by intramuscular route in the gluteal muscle. Treatment was done only once at the beginning of the study;

Group 4. The last three villages were controls and received no further treatment apart from the diminazene aceturate they received at day -42 and day -14 before first intervention (day 0).

Consent was obtained from the farmers before any treatment were applied.

6.3.4. Duration of the study and sample collection

The longitudinal study was conducted over a period of seven months starting in May/June 2004 (baseline survey) and ended in December 2004. During this period animals were monitored for tsetse- and tick-borne diseases, tick infestation, haemoglobin values once every 28 days. The prevalence of tsetse and tick-borne parasites were determined by both microscopy examination of the thick and thin blood smears and PCR analysis (chapter 2). The haemoglobin values were measured using HemoCue Hb 201^{+} analyser (HemoCue AB, Ängelholm, Sweden). See chapter two of this thesis for the details of the methods used. Consent was obtained from the farmers each time samples were collected.

Tsetse traping was not done because they are expensive are heavily biased and lack sensitivity especially for *Glossina morsitans morsitans* and *G. brevipalpis* (Hargroove, 1980). On the plateau in Petauke district only *G. morsitans morsitans* are found in the study area(Van den Bossche *et al,* 2004).

6.3.5. Other useful parameters collected during the study: rainfall and temperature

Data for rainfall and temperature pattern for the area were collected from the Meteorological Department, Lusaka, Zambia for the year 2004. The rainy season in Zambia starts from November and ends in April. During the study in the year 2004 rains started in October and ended in April. The mean monthly rainfall in the area during the study period ranged from 64.2 mm in October when it was lowest to 235.1 mm in January 2004 when it was highest (Table 6.1 and Figure 6.1). Temperature was lowest in June when it was 20.5°C and highest in March when it was 26.2°C.. Meteorological data was obtained from the Department of Meteorology, Lusaka, Zambia

Table 6. 1 Mean monthly rainfall (mm) and temperature (°C) for Petauke District for the year 2004.

	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	Nov	Dec
Rainfall (mm)	235.1	203.4	111.3	104.1	0	0	0	0	0	64.2	103.2	144.8
Temp (°C)	25.4	26	26.2	23.6	21.1	20.5	20.7	21.5	21.5	22.2	22.3	24.1

Source: Meteorological Department, Lusaka, Zambia

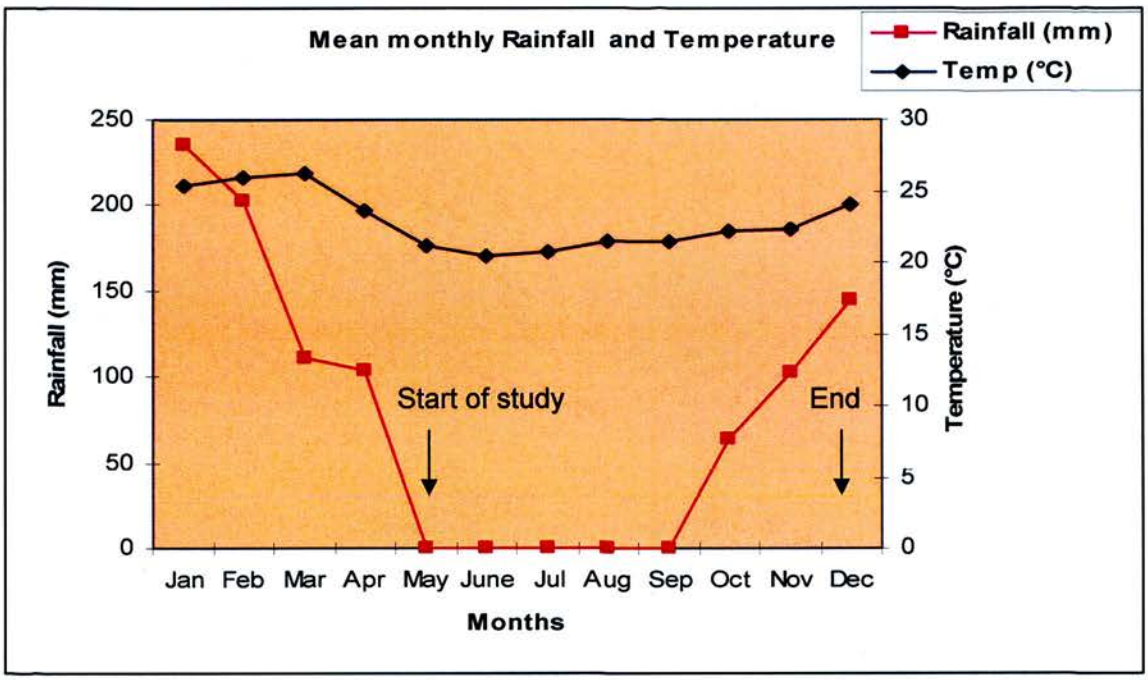


Figure 6. 1 Mean monthly rainfall (mm) and temperature (°C) for Petauke District for the year 2004.

Source: Meterological Department, Lusaka

6.4 Results

6.3.6. Animals sampled monthly during the study period

The number of animals sampled at each of the twelve villages varied throughout the study period. The mean percentage fallout per visit was 28.5% (range: from 18.7% to 36.5%). The numbers that were sampled at each visit and village are tabulated below (Table 6.2). Attendance was not good at Manga but good at at Makale, Mnyeche and Chalokwa.

Table 6. 2 Animal attendance.

Treatment	Village	Baseline day -42	Day -14	Day 0	Day 28	Day 56	Day 84	Day 112	Day 140
Control	Nyamphande	80	50	47	61	47	58	27	58
	Nkhunta	80	59	48	51	54	50	45	32
	Chalokwa	80	70	73	71	67	72	58	67
ISMM	Chipungu	80	75	62	59	54	62	44	41
	Manga	80	60	60	50	38	47	49	47
	Chipembe	80	67	61	55	50	57	52	53
Pour-on	Kasonde	80	57	54	49	54	52	50	56
	Sikwenda	80	59	53	52	54	55	51	51
	Chikuse	78	68	54	51	58	59	52	51
RA	Kasero	80	62	56	54	53	47	50	52
	Mnyeche	80	74	74	61	69	73	73	55
	Makale	80	78	72	61	71	53	57	51
Total attended		**958**	**779**	**714**	**675**	**669**	**685**	**608**	**614**
Did not attend			179	244	283	289	273	350	344
Percentage (%) fallout			18.68	20.47	29.54	30.17	28.50	36.53	35.91

ISMM: isometamidium chloride

RA: Restricted application of deltamethrin to legs, belly and ears

6.3.7. Trypanosomiases

6.3.7.1. Monthly incidence of trypanosomes in cattle from the 12 villages in Petauke District.

The incidence of trypanosomiasis in all the 12 villages was very low after the animals were treated with a double dose of diminazene aceturate. Only four villages recorded trypanosomes infections by either microscopy or PCR at any time after the animals were treated after the second dose of diminazene aceturate on day -14. Tables 6.3a and 6.3b show the number of animals that were sampled and the number that were positive by microscopy or PCR respectively. The villages where animals positive for trypanosomes were found after day 0 were Makale, Chipungu, Kasero and Chipembe (Table 6.3a and 6.3b). Makale the village that lies near the South Luangwa National Park had the highest number of infections throughout the study period.

Table 6. 3a Monthly incidence (%) and species of trypanosomes in cattle under various treatment regimes (microscopy results).

Treatment	Villages	Day(0)	Day(28)	Day(56)	Day(84)	Day(112)	Day(140)
Control	Chalokwa	0	0	0	0	0	0
	Nkhunta	0	0	0	0	0	0
	Nyamphande	0	0	0	0	0	0
ISMM	Manga	0	0	0	0	0	0
	Chipembe	0	0	0	0	0	0
	Chipungu	0	0	0	0	2/44 = **4.55%** (2; 1*Tc* & 1*Tc*/*Tb*)	0
Pour-on	Sikwenda	0	0	0	0	0	0
	Kasonde	0	0	0	0	0	0
	Chikuse	0	0	0	0	0	0
RA	Mnyeche	0	0	0	0	0	0
	Kasero	0	0	0	0	2/47 = **4.26%** (2*Tc*)	0
	Makale	0	2/61 = **3.28%** (2*Tc*)	6/71 = **8.45%** (6*Tc*)	1/53 = **1.87%** (1*Tc*)	5/57 = **8.77%** (5; 3*Tc*, 2*Tc*/*Tb*)	0

ISMM = isometamidium chloride RA = Restricted application *T b = T. brucei* *Tc = T. congolense*

Table 6. 3b Monthly incidence (%) and trypanosome species of trypanosomes in cattle under various treatment regimes (PCR results).

Treatment	Villages	Day 0	Day 28	Day 56	Day 84	Day 112	Day 140
Control	Chalokwa	0	0	0	0	0	0
	Nkhunta	0	0	0	0	0	0
	Nyamphande	0	0	0	0	0	0
ISMM	Manga	0	0	0	0	0	0
	Chipembe	0	3/55 = **5.45%** (3*Tc*)	0	0	2/52 = **2.85%** (2*Tv*)	0
	Chipungu	0	0	2/54 = **3.70%** (1*Tc*)	0	0	0
Pour-on	Sikwenda	0	0	0	0	0	0
	Kasonde	0	0	0	0	0	0
	Chikuse	0	0	0	0	0	0
RA	Mnyeche	0	0	0	0	0	0
	Kasero	0	0	0	0	0	0
	Makale	0	0	2/71 = **2.82%** (2*Tv*)	3/53 = **5.67%** (3; 2*Tc* & 1*Tv*)	0	0

ISMM = isometamidium chloride RA =Restricted application *Tb* = *T. brucei*, *Tc* = *T. congolense* and *Tv* = *T. vivax*

6.3.8. Tick-borne diseases

6.3.8.1. Monthly prevalence of *Theileria* parasites infection detected in cattle under various treatment regimes

Theileria parasites were detected in cattle in most of the villages on most occasions of sampling and the prevalence varied from village to village and month to month (Tables 6.4a and 6.4b).

The mean prevalence of *Theileria parva* diagnosed by PCR in the RA treated villages was higher (22.5%; 95%CI: 17.0% to 29.3%) during the first few months and low (0%; 95%CI: 0% - 4.3%) towards the end of the study. In the pour-on villages the mean prevalence was also higher (18.6%; 95%CI: 12.7% to 26.3%) at the beginning of the trial and low (0%; 95%CI: 0 % to 5%) at the end. In the ISMM villages the mean prevalence was almost the same at the beginning (12.3%; 95%CI: 8% to 18.5%) and at the end (11.2%; 95%CI: 6% to 20%). In the control villages the mean prevalence was lower (15.5%; 95%CI: 10.3% to 22.7%) at the beginning and high (20.9%; 95%CI: 13.7% to 30.7%) at the end of the trial (Tables 6.4a).

The mean prevalence of *Theileria* parasites diagnosed by Microscopic examination was higher in all the four treatment groups at day 56 and day 114 after the first intervention (Table 6.4b). During these two peaks, at day 56 post first intervention (first peak), the mean prevalence were higher in the RA (30.6%; 95%CI: 24.2% to 37.9%) and pour-on groups (26.7%; 95%: 20.2% to 34.3%) but lower in the control (16.3%; 95%CI: 11.0% to 23.4%) and ISMM groups (18.6%; 95%CI: 12.5% to 26.7%). However towards the end of the trial (second peak: day 114) the mean prevalence were higher in the control (22.4%; 95CI: 14.8% to 32.3%) and ISMM groups (18.2%; 95%CI: 11.8% - 26.9%) and low in the pour-on (11.6%; 95%CI: 6.80% to 19.3%) and RA (13%; 95%CI: 8.60% to 21.7%) treated groups (Table6.3b).

Table 6. 4a Prevalence of *Theileria* parasites infections detected by PCR in cattle under various treatment regimens.

		Prevalence (I)					
Treatment group	**Village**	**Day 0**	**Day 28**	**Day 56**	**Day 84**	**Day 112**	**Day 140**
		July	**August**	**September**	**October**	**November**	**December**
Control	Chalokwa	$0.0769_{(4/52)}$	$0.1860_{(8/43)}$	$0.0571_{(2/35)}$	$0.0833_{(3/36)}$	$0.0385_{(1/26)}$	$0.357_{(10/28)}$
	Nkhunta	$0.0750_{(10/35)}$	$0.4400_{(11/25)}$	$0.1500_{(3/20)}$	$0.0357_{(1/28)}$	$0.0000_{(0/14)}$	$0.0000_{(0/15)}$
	Nyamphande	$0.1429_{(6/42)}$	$0.1042_{(5/48)}$	$0.0833_{(3/36)}$	$0.0476_{(2/42)}$	$0.0000_{(0/11)}$	$0.1860_{(8/43)}$
Subtotals		$0.155_{(20/129)}$	$0.2069_{(24/116)}$	$0.0879_{(8/91)}$	$0.0566_{(6/106)}$	$0.0196_{(1/51)}$	$0.2093_{(18/86)}$
Pour-on	Kasonde	$0.1944_{(7/36)}$	$0.2414_{(7/29)}$	$0.1333_{(4/30)}$	$0.0000_{(0/27)}$	$0.0000_{(0/27)}$	$0.0000_{(0/27)}$
	Sikwenda	$0.2857_{(10/35)}$	$0.4400_{(11/25)}$	$0.1500_{(3/20)}$	$0.0357_{(1/28)}$	$0.0000_{(0/14)}$	$0.0000_{(0/15)}$
	Chikuse	$0.1132_{(6/53)}$	$0.2222_{(10/45)}$	$0.0732_{(3/41)}$	$0.0526_{(2/38)}$	$0.0000_{(0/31)}$	$0.0000_{(0/31)}$
Subtotals		$0.1855_{(23/124)}$	$0.2828_{(28/99)}$	$0.1099_{(10/91)}$	$0.0322_{(3/93)}$	$0.0000_{(0/72)}$	$0.0000_{(0/73)}$
ISMM	Manga	$0.1395_{(6/43)}$	$0.3226_{(10/31)}$	$0.1111_{(2/18)}$	$0.0476_{(1/21)}$	$0.0000_{(0/23)}$	$0.0476_{(1/21)}$
	Chipembe	$0.1509_{(8/53)}$	$0.2683_{(11/41)}$	$0.0345_{(1/29)}$	$0.0303_{(1/33)}$	$0.0000_{(0/29)}$	$0.1034_{(3/29)}$
	Chipungu	$0.0862_{(5/58)}$	$0.1000_{(5/50)}$	$0.0250_{(1/40)}$	$0.0417_{(2/48)}$	$0.0313_{(1/32)}$	$0.1667_{(5/30)}$
Subtotals		$0.1233_{(19/154)}$	$0.2131_{(26/122)}$	$0.0460_{(4/87)}$	$0.0392_{(4/102)}$	$0.0119_{(1/84)}$	$0.1125_{(9/80)}$
RA	Kasero	$0.2941_{(10/34)}$	$0.1481_{(4/27)}$	$0.0000_{(0/19)}$	$0.0556_{(1/18)}$	$0.1579_{(3/19)}$	$0.0000_{(0/18)}$
	Mnyeche	$0.2571_{(18/70)}$	$0.1750_{(7/40)}$	$0.0488_{(2/41)}$	$0.0233_{(1/43)}$	$0.0000_{(0/41)}$	$0.0000_{(0/29)}$
	Makale	$0.1594_{(11/69)}$	$0.1400_{(7/50)}$	$0.0577_{(3/52)}$	$0.0000_{(0/37)}$	$0.0000_{(0/42)}$	$0.0000_{(0/38)}$
		$0.2254_{(39/173)}$	$0.1538_{(18/117)}$	$0.0446_{(5/112)}$	$0.0204_{(2/98)}$	$0.0294_{(3/102)}$	$0.0000_{(0/85)}$

ISMM = isometamidium chloride, Numbers in brackets = number of new positive animals over number at risk. RA = Restricted application of insecticide

Table 6. 4b Prevalence of *Theileria* parasites infections detected by microscopy in cattle under various treatment regimens.

		Prevalence (I)					
Treatment Group	**Village**	**Day 0 July**	**Day 28 August**	**Day 56 September**	**Day 84 October**	**Day 112 November**	**Day 140 December**
Control	**Chalokwa**	$0.0484_{(3/62)}$	$0.0882_{(6/68)}$	$0.0833_{(4/48)}$	$0.0400_{(2/50)}$	$0.2703_{(10/37)}$	$0.0294_{(1/34)}$
	Nkhunta	$0.0682_{(3/44)}$	$0.1556_{(7/45)}$	$0.2750_{(11/40)}$	$0.0000_{(0/27)}$	$0.1923_{(5/26)}$	$0.0000_{(0/18)}$
	Nyamphande	$0.0000_{(0/46)}$	$0.0167_{(1/60)}$	$0.1489_{(7/47)}$	$0.0000_{(0/52)}$	$0.1818_{(4/22)}$	$0.0417_{(2/48)}$
Subtotals		$0.0395_{(6/152)}$	$0.0809_{(14/173)}$	$0.1630_{(22/135)}$	$0.0155_{(2/129)}$	$0.2235_{(19/85)}$	$0.0300_{(3/100)}$
Pour-on	**Kasonde**	$0.0588_{(3/51)}$	$0.0444_{(2/45)}$	$0.1875_{(9/48)}$	$0.0238_{(1/42)}$	$0.0857_{(3/35)}$	$0.0270_{(1/37)}$
	Sikwenda	$0.0000_{(0/50)}$	$0.0816_{(4/49)}$	$0.1489_{(7/47)}$	$0.0000_{(0/42)}$	$0.0513_{(2/39)}$	$0.0000_{(0/37)}$
	Chikuse	$0.0377_{(2/53)}$	$0.0000_{(0/48)}$	$0.4364_{(24/55)}$	$0.0000_{(0/33)}$	$0.2414_{(7/29)}$	$0.0455_{(1/22)}$
Subtotals		$0.0325_{(5/154)}$	$0.0422_{(6/142)}$	$0.2667_{(40/150)}$	$0.0085_{(1/117)}$	$0.1165_{(12/103)}$	$0.0208_{(2/96)}$
ISMM	**Manga**	$0.0755_{(4/53)}$	$0.0500_{(2/40)}$	$0.2759_{(8/29)}$	$0.0333_{(1/30)}$	$0.1875_{(6/32)}$	$0.0333_{(1/30)}$
	Chipembe	$0.0714_{(4/56)}$	$0.0000_{(0/47)}$	$0.1190_{(5/42)}$	$0.0444_{(2/45)}$	$0.1316_{(5/38)}$	$0.0278_{(1/36)}$
	Chipungu	$0.0000_{(0/48)}$	$0.0000_{(0/48)}$	$0.1905_{(8/42)}$	$0.0000_{(0/41)}$	$0.2414_{(7/29)}$	$0.0000_{(0/21)}$
Subtotals		$0.0510_{(8/157)}$	$0.0148_{(2/135)}$	$0.1858_{(21/113)}$	$0.0259_{(3/116)}$	$0.1818_{(18/99)}$	0.0230
RA	**Kasero**	$0.0185_{(1/54)}$	$0.0385_{(2/52)}$	$0.2708_{(13/48)}$	$0.0294_{(1/34)}$	$0.2059_{(7/34)}$	$0.0345_{(1/29)}$
	Mnyeche	$0.1014_{(7/69)}$	$0.0000_{(0/55)}$	$0.3770_{(23/61)}$	$0.0263_{(1/38)}$	$0.1579_{(6/38)}$	$0.0000_{(0/28)}$
	Makale	$0.0156_{(1/64)}$	$0.0000_{(0/54)}$	$0.2623_{(16/61)}$	$0.0000_{(0/34)}$	$0.0556_{(2/36)}$	$0.0000_{(0/33)}$
Subtotals		$0.0482_{(9/187)}$	$0.0124_{(2/161)}$	$0.3059_{(52/170)}$	$0.0189_{(2/106)}$	$0.1389_{(15/108)}$	$0.0111_{(1/90)}$

ISMM = isometamidium chloride. Numbers in brackets = number of new positive animals over number at risk. RA = Restricted application of insecticide

6.3.8.2. Cumulative prevalence of *Theileria* parasites in cattle

6.3.8.3. Cumulative prevalence of *Theileria* parasites in cattle diagnosed by PCR

The cumulative prevalence of *Theileria* parasites in cattle diagnosed by PCR is shown in Table 6.5a and Figure 6.2a. The cumulative prevalence of animals positive for *Theileria parva* rose continuously throughout the study in the control villages and those villages where animals were treated with isometamidium chloride. In villages where cattle were treated with insecticide in the form of pour-on or spray, the cumulative prevalence increased initially but after the third application the number stabilized (Table 6.5a and Figure 6.2a).

6.3.8.4. Cumulative prevalence of *Theileria* parasites in cattle diagnosed by microscopy.

The cumulative number of positive animals for *Theileria species* diagnosed by microscopic examination followed the same pattern as observed by PCR amplification for *Theileria parva* with little variation although there was a sharp increase in the numbers two months after the start of the application of insecticide. After two months in two of the three pour-on villages and two out of three spray villages the number stabilized; the exceptions were Mnyeche (spray village) and Chikuse (pour-on village). (Table 6.5b and Figure 6.2b).

Table 6. 5a Cumulative prevalence (%) of *Theileria* parasites in cattle diagnosed by PCR.

		Days					
Treatment	**Villages**	**0**	**28**	**56**	**84**	**112**	**140**
Control	Chalokwa	7.69	26.30	32.01	40.34	44.19	79.91
	Nkhunta	7.50	17.26	34.76	34.76	44.76	44.76
	Nyampande	14.29	24.70	33.04	37.80	37.80	56.40
Pour-on	Kasonde	19.44	43.58	56.92	56.92	56.92	56.92
	Sikwenda	28.57	72.57	87.57	91.14	91.14	91.14
	Chikuse	11.32	33.54	40.86	46.12	46.12	46.12
ISMM	Manga	13.95	46.21	57.32	62.08	62.08	66.85
	Chipungu	8.62	18.62	21.12	25.29	28.41	45.08
	Chipembe	15.09	41.92	45.37	48.40	48.40	58.75
RA	Kasero	29.41	44.23	44.23	49.78	65.57	65.57
	Mnyeche	25.71	43.21	48.09	50.42	50.42	50.42
	Makale	15.94	29.94	35.71	35.71	35.71	35.71

ISMM = isometamidium chloride RA = restricted application of insecticide.

Table 6. 5b Cumulative prevalence (%) of *Theileria* parasites in cattle diagnosed by microscopy.

		Days					
Treatment	**Village**	**0**	**28**	**56**	**84**	**112**	**140**
Control	Chalokwa	4.84	15.18	23.53	27.52	54.54	57.49
	Nkhunta	6.82	22.37	49.87	49.87	69.10	69.10
	Nyampande	0.00	1.67	16.56	16.56	34.74	34.74
Pour-on	Kasonde	5.88	10.33	29.08	31.46	40.03	42.73
	Sikwenda	0.00	8.16	23.06	23.06	28.19	28.19
	Chikuse	3.77	3.77	47.41	47.41	71.55	76.09
ISMM	Manga	7.55	12.55	40.13	43.47	62.22	65.55
	Chipungu	0.00	0.00	19.05	19.05	43.19	43.19
	Chipembe	7.14	7.14	19.05	23.49	36.65	39.43
RA	Kasero	1.85	5.70	32.78	35.72	56.31	59.76
	Mnyeche	10.14	10.14	47.85	50.48	66.27	66.27
	Makale	1.56	1.56	27.79	27.79	33.35	33.35

ISMM = isometamidium chloride RA = restricted application of insecticide.

Figure 6. 2a Cumulative prevalence of *Theileria* parva diagnosed by PCR under treatment regimes.

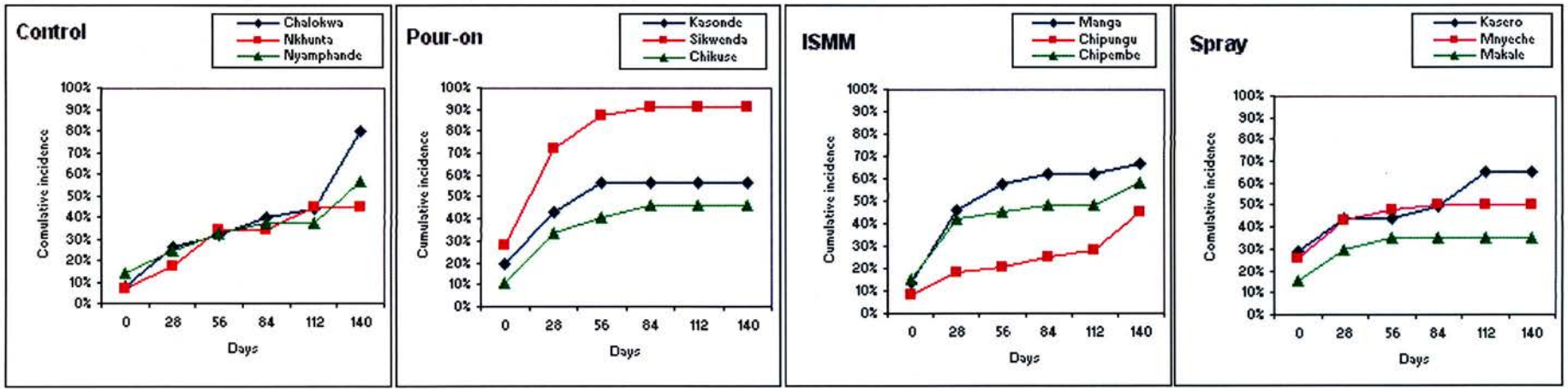
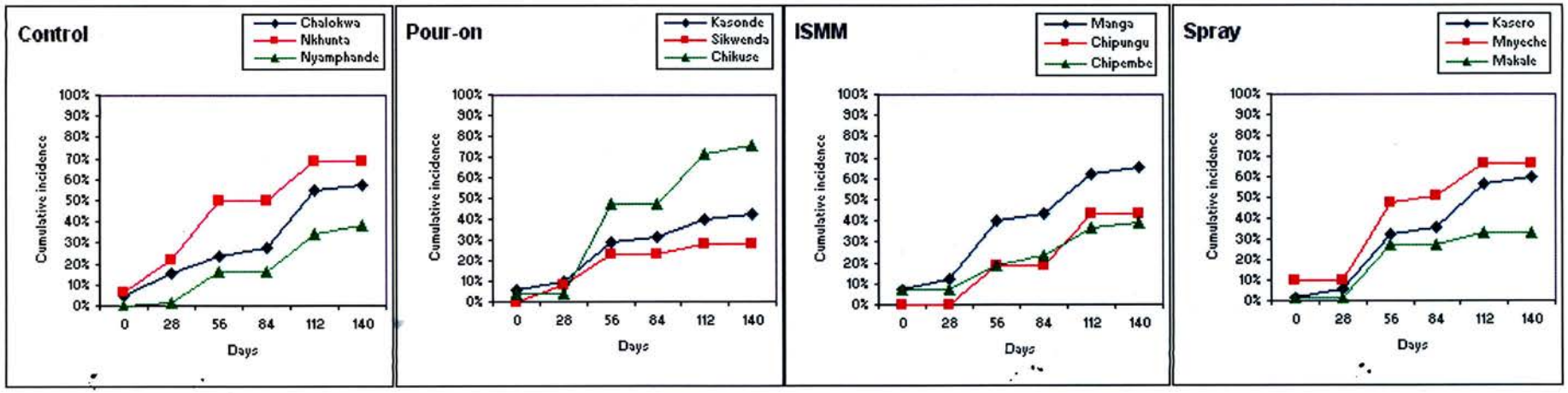


Figure 6. 2b Cumulative prevalence of *Theileria* parasites diagnosed by microscopy in cattle under various treatment regimes.



6.3.8.5. Proportion of animals that survived *Theileria* infection under various treatment regimes.

6.3.8.5.1. Proportion of animals that survived *Theileria parva* infection diagnosed by PCR under various treatment regimes.

In the control groups 43 % (95% Confidence Interval: from 25% to 61%) of the animals at Chalokwa village survived without infection up to 140 days. At Nkhunta 67 % (95% CI: from 43% to 91%) of the animals survived without infection while at Nyamphande 64% (95% CI: from 50% to 78%) were still negative up to 140 days (Table 6.4). The numbers of cattle censored at these control villages were as follows; at Chalokwa village out of the initial 52 cattle at risk 24 were censored, at Nkhunta it was 23 out of 40 and at Nyamphande was 18 out of 42 cattle. Censored observations are those animals that became positive or were lost during the course of the study.

In the three pour-on villages 66% (95% CI: from 49% to 83%) of the cattle remained uninfected up to day 140 at Kasonde while at Sikwenda 46% (95% CI: from 49% to 83%) remained negative for the parasites for the same duration. At Chikuse village 68% (955 CI: from 21% to 71%) of the cattle were negative up to 140 days (Table 6.6). When the survival proportional results of the three pour-on villages were pooled and compared with the pooled results of the three control villages the increase in the survival proportional was only 2% with a 95% confidence interval from -12.6% to 16%. Eighteen (18) out of the 36 initial cattle that were at risk were censored at Kasonde. At Sikwenda 10 out of 35 were censored while at Chikuse 32 out of 53 were censored.

The ISMM treatment villages (Table 6.6) showed that at Manga 55% (95% CI: from 33% to 76%) of the cattle remained free of infection for 140 days while at Chipembe village it was 61% (95% CI: from 43% to 79%) and at Chipungu was 68% (95% CI: 51% to 85%). When the survival proportional results of the three ISMM treated villages were pooled and compared with the pooled results of the three control villages the increase in the survival proportional was only 3.3% (95% CI: from -11.7% to 18%). At Manga out of the initial number of 43 cattle that were at risk 23

were censored and at Chipembe it was 29 out of 53. At Chipungu 39 out of 58 cattle were censored.

In the RA treated villages 81% (95% CI: from 63% to 99%) of the cattle survived without infection at Kasero while at Mnyeche 77% (95% CI: from 62% to 92%) remained uninfected for 140 days. At Makale 68% (95% CI: from 53% to 83%) remained free of infection during the same period (Table 6.6). When the survival proportional results of the three RA villages were pooled and compared with the pooled results of the three control villages the increase in the proportional survival was 17.5% (95% CI: from 3.2% to 31%). Sixteen (16) cattle out of 34 that were initially at risk were censored at Kasero and at Mnyeche 42 out of 70 cattle that were at risk were censored. At Makale 48 out of 69 that were at risk were censored.

Table 6. 6 Proportion of animals that survived *Theileria* parasites infection diagnosed by PCR under various treatment regimes

		Days					
Treatment	**Villages**	**0**	**28**	**56**	**84**	**112**	**140**
Control	Chalokwa	1	0.81	0.77	0.70	0.67	0.43
	Nkhunta	1	0.90	0.74	0.74	0.67	0.67
	Nyampande	1	0.90	0.82	0.78	0.78	0.64
Pour-on	Kasonde	1	0.76	0.66	0.66	0.66	0.66
	Sikwenda	1	0.56	0.48	0.46	0.46	0.46
	Chikuse	1	0.78	0.72	0.68	0.68	0.68
ISMM	Manga	1	0.68	0.60	0.57	0.57	0.55
	Chipungu	1	0.90	0.88	0.84	0.81	0.68
	Chipembe	1	0.73	0.71	0.69	0.69	0.61
RA	Kasero	1	0.86	0.81	0.81	0.81	0.81
	Mnyeche	1	0.83	0.78	0.77	0.77	0.77
	Makale	1	0.85	0.85	0.80	0.68	0.68

ISMM = isometamidium chloride RA = restricted application of insecticide

6.3.8.5.2. Proportion of *animals that survived* Theileria parasites diagnosed by microscopy under various treatment regimes

In the control groups 56% (CI: 39% - 73%) of the animals at Chalokwa village survived without infection up to 140 days. At Nkhunta 49% (CI: 26% - 72%) of the animals survived without infection while at Nyamphande 66% (CI: 53% - 79%) were still negative up to 140 days (Table 6.7). The numbers of cattle censored at these control villages were as follows; at Chalokwa village out of the initial 62 cattle at risk 36 were censored, at Nkhunta it was 18 out of 44 and at Nyamphande was 32 out of 46. Censored observations are those animals that became positive or were lost during the course of the study.

In the three pour-on treatment villages 67% (95% CI: from 52% to 82%) of the cattle remained uninfected up to day 140 at Kasonde while at Sikwenda 74% (95% CI: from 60% to 88%) remained negative for the parasites for the same duration. At Chikuse village 41% (95% CI: from 20% to 62%) of the cattle were negative up to 140 days (Table 6.7). When the survival proportional results of the three pour-on villages were pooled and compared with the pooled results of the three control villages the increase in the survival proportional was about 4% with a 95% confidence interval from -11.9% to 19.5%. Thirty two (32) out of the 51 initial cattle that were at risk were censored at Kasonde. At Sikwenda 37 out of 50 were censored while at Chikuse 19 out of 53 were censored.

The ISMM treatment villages (Table 6.7) showed that at Manga 52% (95% CI: 34% from 70%) of the cattle remained free of infection for 140 days while at Chipembe village it was 71% (95% CI: from 56% to 86%) and at Chipungu was 61% (CI: from 40% to 82%). When the survival proportional results of the three villages were pooled and compared with the pooled results of the three control villages the increase in the survival proportional was 5% with a 95% confidence interval from -9.5% to 18.7%. At Manga out of the initial number of 53 cattle that were at risk 31 were censored and at Chipembe it was 36 out of 56. At Chipungu 33 out of 48 cattle were censored.

In the RA treatment villages 52% (95% CI: from 34% to 70%) of the cattle survived without infection at Kasero while at Mnyeche 51% (95% CI: 32% to 70%) remained uninfected for 140 days. At Makale 69% (95% CI: from 54% to 86%) remained free of infection during the same period (Table 6.7). When the survival proportional results of the three RA treatment villages were pooled and compared with the pooled results of the three control villages the increase in the survival proportional was only 1% with a 95% confidence interval from -13.6% to 14.7%. Twenty-nine (29) cattle out of 54 that were initially at risk were censored at Kasero and at Mnyeche 34 out of 69 cattle that were at risk were censored. At Makale 45 out of 64 were censored.

Table 6. 7 Proportion of animals that survived *Theileria* parasites infection diagnosed by microscopy under various treatment regimes

		Days					
Treatment	**Villages**	**0**	**28**	**56**	**84**	**112**	**140**
Control	Chalokwa	1	0.90	0.82	0.79	0.58	0.56
	Nkhunta	1	0.84	0.61	0.61	0.49	0.49
	Nyampande	1	0.98	0.84	0.84	0.68	0.66
Pour-on	Kasonde	1	0.96	0.78	0.76	0.69	0.67
	Sikwenda	1	0.92	0.78	0.78	0.74	0.74
	Chikuse	1	1	0.56	0.56	0.43	0.41
ISMM	Manga	1	0.95	0.69	0.67	0.54	0.52
	Chipungu	1	1	0.81	0.81	0.61	0.61
	Chipembe	1	1	0.88	0.84	0.73	0.71
RA	Kasero	1	0.96	0.70	0.68	0.54	0.52
	Mnyeche	1	1	0.62	0.61	0.51	0.51
	Makale	1	1	0.74	0.74	0.70	0.70

ISMM = isometamidium chloride RA = restricted application of insecticide

6.3.8.6. Mortality of animals and the number that died of *Theileria* parasites during the study

A total of 54 animals (Table 6.8) died during the course of the trial. Fourteen (14) animals were reported to have died between day -42 (May/June) and day 0 (July), 12 at day 28 (August), 5 at day 56 (September), 12 at day 84 (October) and 11 at day 112 (November). Most of the animals that died were at Kasero (18/80 = 22.5%) and Sikwenda (11/80 = 13.75%).

Out of the 54 animals that died during the study, 21 (38.89%) died as a result of *Theileria* parasites (Table 6.8). The diagnosis was based on either microscopic examination of the thick and thin blood smears or PCR and clinical signs farmers observed in the animals just before they died. The common clinical signs observed in all the 21 animals by the farmers were lacrimation, nasal discharge, lymphnode enlargement and no appetite. Two (9.52%) out of 21 were positive only on microscopy examination and 12 (57.14%) out of 21 were positive by PCR. Seven (33.33%) out of 21 came out positive by both methods. Most of the animals that died with *Theileria* parasites were in the younger age group (Table 6.9 and Figure 6.3). The number of deaths in the older animals was low (Table 6.9 and Figure 6.3).

Table 6. 8 Mortality of cattle and the number that died of *Theileria* parasites during the study.

Treatment	Village	Day 0	Day 28	Day 56	Day 84	Day 112	Day 140	Total
Control	Nyamphande	0	$3_{(1)}$	0	0	0	0	**$3_{(1)}$**
	Nkhunta	0	$1_{(0)}$	0	$2_{(1)}$	0	0	**$3_{(1)}$**
	Chalokwa	0	0	0	$1_{(0)}$	0	0	**$1_{(0)}$**
ISMM	Chipungu	0	0	0	0	0	0	**0**
	Manga	$1_{(1)}$	$2_{(0)}$	0	0	$4_{(2)}$	0	**$7_{(3)}$**
	Chipembe	$1_{(0)}$	0	$1_{(0)}$	0	0	0	**$2_{(0)}$**
Pour-on	Kasonde	0	0	$2_{(2)}$	$2_{(2)}$	0	0	**$4_{(4)}$**
	Sikwenda	$6_{(3)}$	$5_{(4)}$	0	0	0	0	**$11_{(7)}$**
	Chikuse	0	0	0	0	0	0	**0**
RA	Kasero	$3_{(0)}$	$1_{(0)}$	$2_{(0)}$	$6_{(3)}$	$6_{(1)}$	0	**$18_{(4)}$**
	Mnyeche	$3_{(0)}$	0	0	$1_{(0)}$	$1_{(1)}$	0	**$5_{(1)}$**
	Makale	0	0	0	0	0	0	**0**
Total		**$14_{(4)}$**	**$12_{(5)}$**	**$5_{(2)}$**	**$12_{(6)}$**	**$11_{(4)}$**	**0**	**$54_{(21)}$**

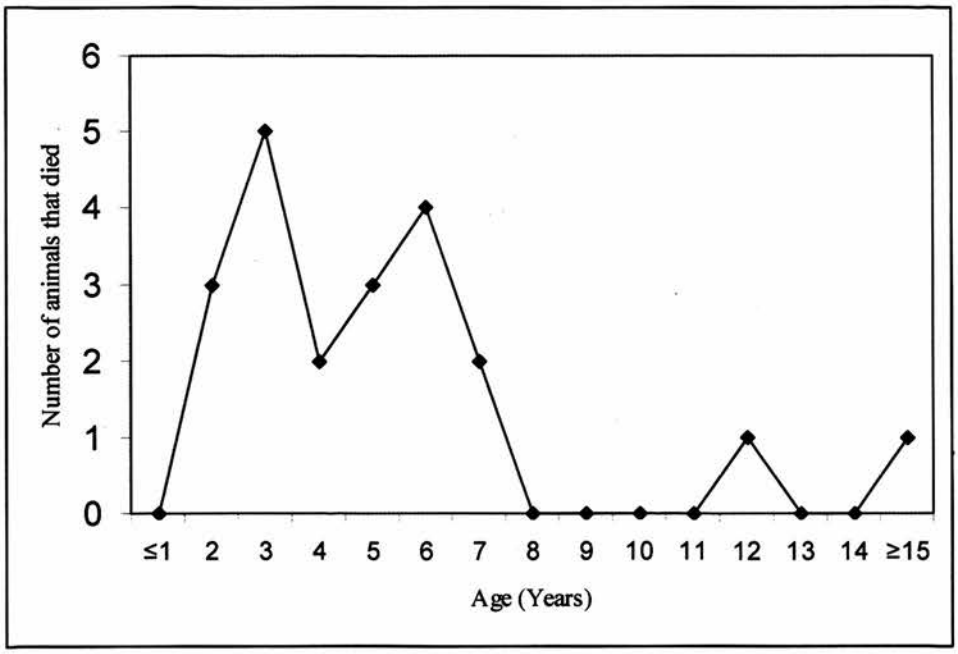
ISMM = isometamidium chloride. RA = restricted application of insecticide

Numbers in the brackets are animals with *Theileria* parasites.

Table 6. 9 Ages of cattle dying of *Theileria* parasites by age group

	Age (years) of the animals															
Age (yrs)	≤1	2	3	4	5	6	7	8	9	10	11	12	13	14	≥15	Total
Number of deaths	0	3	5	2	3	4	2	0	0	0	0	1	0	0	1	21
Percentage (%)	0	14.28	23.81	9.52	23.81	19.05	9.52	0	0	0	0	4.76	0	0	4.76	

Figure 6. 3 Ages of cattle dying from *Theileria* parasites by age group



6.3.8.7. *Anaplasma*

Very few cattle were diagnosed with *Anaplasma* in the area by microscopy screening of the thick and thin smears stained with Giemsa stain (Table 6.10). In total thirty-seven animals were found with *Anaplasma* during the study period. Slightly high numbers were seen in June (day -14) and in November (Day 112).

Table 6. 10 Number of cattle with *Anaplasma* parasites.

Treatment	Village	Baseline (day -42)	Day -14	Day 0	Day 28	Day 56	Day 84	Day 112	Day 140
Control	Nyamphande	0	3	0	1	0	0	0	1
	Nkhunta	0	0	0	0	1	0	1	1
	Chalokwa	0	1	1	0	0	1	0	0
Subtotal		**0**	**4**	**1**	**1**	**1**	**1**	**1**	**2**
ISMM	Chipungu	2	2	0	0	0	0	0	0
	Manga	0	0	0	0	0	1	1	0
	Chipembe	0	0	0	0	0	0	0	0
Subtotal		**2**	**2**	**0**	**0**	**0**	**1**	**1**	**0**
Pour-on	Kasonde	1	0	0	0	0	0	0	1
	Sikwenda	1	2	0	0	0	0	2	0
	Chikuse	1	1	1	0	0	0	3	0
Subtotal		**3**	**3**	**1**	**0**	**0**	**0**	**5**	**1**
RA	Kasero	0	0	0	0	0	1	0	0
	Mnyeche	0	2	0	0	0	1	1	0
	Makale	0	1	0	0	0	0	1	0
Subtotal		**0**	**3**	**0**	**0**	**0**	**2**	**2**	**0**
Grand total		**5**	**12**	**2**	**1**	**1**	**4**	**9**	**3**

ISMM = isometamidium chloride RA = restricted application of insecticide.

6.3.8.8. *Babesia*

Cattle found with *Babesia* parasites were very few throughout the study period (Table 6.11). Only ten cases were diagnosed by microscopy examination of the thick and thin blood smears stained with Giemsa. Four cases were in May/June (day -42), two in June (day -14). One case was in September (day 56) and two in November (day 112).

Table 6. 11 Number of cattle with *Babesia* parasites.

Treatment	Village	Baseline (day -42)	Day -14	Day 0	Day 28	Day 56	Day 84	Day 112	Day 140
Control	Nyamphande	0	0	0	0	0	0	0	0
	Nkhunta	0	0	0	0	1	0	0	0
	Chalokwa	2	0	0	0	0	0	0	0
Subtotal		2	0	0	0	1	0	0	0
ISMM	Chipungu	0	0	0	0	0	0	0	0
	Manga	0	0	0	0	0	0	0	0
	Chipembe	0	0	0	0	0	0	0	0
Subtotal		0	0	0	0	0	0	0	0
Pour-on	Kasonde	2	0	0	0	0	0	0	0
	Sikwenda	0	2	0	0	0	0	0	0
	Chikuse	0	0	0	0	0	0	2	0
Subtotal		2	2	0	0	0	0	2	0
RA	Kasero	0	0	0	0	0	0	0	0
	Mnyeche	0	0	0	0	0	0	0	0
	Makale	0	0	0	0	0	0	0	0
Subtotal		0	0	0	0	0	0	0	0
Grand total		**4**	**2**	**0**	**0**	**1**	**0**	**2**	**0**

ISMM = isometamidium chloride RA = restricted application of insecticide.

6.3.8.9. Monthly tick burden on cattle under various treatment regimes

6.3.8.9.1. Proportion of cattle in each tick (*Amblyomma* species) burden category under various treatment regimes.

Isometamidium chloride (ISMM) treated villages compared with control villages

The proportion of animals in the zero category (no ticks) in the ISMM treated villages were not statistically significant from the proportion of animals that were in the control villages (Figure 6.4 and Appendix 8 [Table 8.1a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the ISMM treated villages and control villages was also not statistically significant ($p > 0.05$) from day zero to day 140 (Figure 6.4 and Appendix 8, [Table 8.1b]). The difference was only significant at day 28 post treatment (95% CI = from -0.1486 to -0.0075; d.f. = 1; p-value= 0.0278; $\chi^2 = 5.06$). There was no difference between the control villages and isometamidium chloride villages in the medium and high categories (Figure 6.4 and Appendix 8, Tables 8.1c and 8.1d]).

Pour-on treated villages compared with control villages

At day 56, day 84 and day 140 proportion of animals in the zero category (no ticks) in the pour-on treated villages were high and statistically significant ($p < 0.05$) from the proportion of animals that were in the control villages (Figure 6.4 and Appendix 8 [Table 8.2a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the pour-on treated villages and control villages were statistically significant ($p < 0.05$) at day 56, day 112 and day 140 (Figure 6.4 and Appendix 8 [Table 8.2b]) The proportion was high in the pour-on villages.

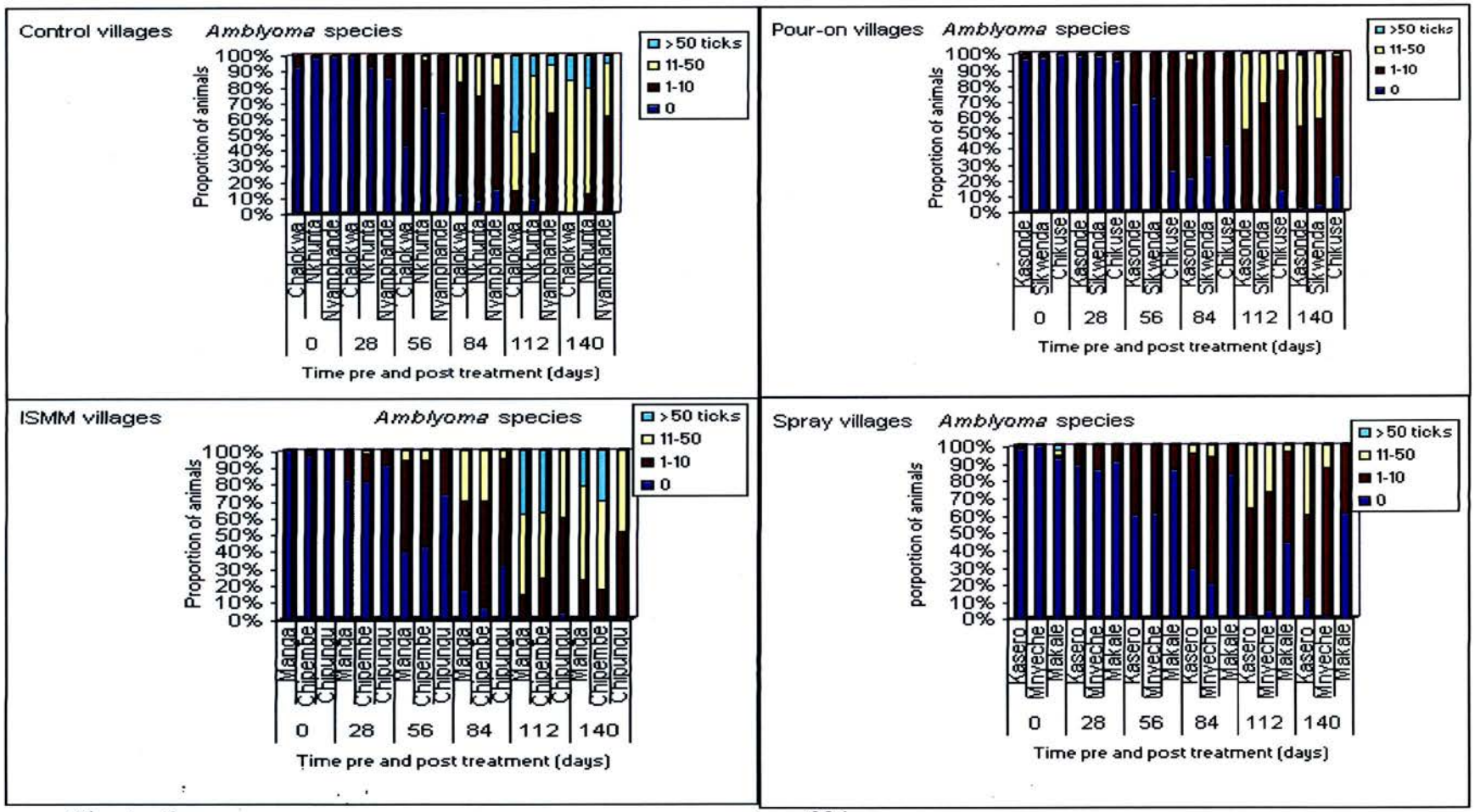
In the medium category (11-50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the pour-on villages at day 84 and day 140 (Figure 6.4 and Appendix 8 [Table 8.2c]). In the high category (> 50 ticks) the proportions of animals were high and statistically significant ($p < 0.05$) in the control villages compared with the pour-on villages (Figure 6.4 and Appendix 8 [Table 2d]).

Restricted application of insecticide (RA) treated villages compared with control villages

From 56 to day 140 proportion of animals in the zero category (no ticks) in the RA treated villages were high and statistically significant ($p < 0.05$) from the proportion of animals that were in the control villages (Figure 6.4 and Appendix 8 [Table 8.3a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the RA treated villages and control villages were statistically significant ($p < 0.05$) also from day 56 to day 140 (Figure 6.4 and Appendix 8 [Table 8.3b]).). The proportion was high in the RA villages.

In the medium category (11-50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the RA villages from day 84 to day 140 (Figure 6.4 and Appendix 8 [Table 8.3c]).). In the high category (> 50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the spray villages at day 112 and day 140 (Figure 6.4 and Appendix 8 [Table 8.3d]).

Figure 6. 4 Proportion of animals with tick burden (*Amblyomma* ticks) in the categories 0= zero ticks, category 1 = 1-10 ticks, category 2= 11-50 ticks, category 3 = > 50 ticks under various treatment regimes. Spray = restricted application of insecticide (RA)



6.3.8.9.2. Proportion of cattle in each tick (*Boophilus* species) burden category under various treatment regimes

Isometamidium chloride (ISMM) treated villages compared with control villages

The proportion of animals in the zero category (no ticks) in the ISMM treated villages were not significantly different from the proportion of animals that were in the control villages (Figure 6.5 and Appendix 9 [Table 9.1a]). It was statistically different only at day 84 (95% CI = from 0.0743 to 0.2690; d.f. = 1; p-value= 0.004; χ^2 = 12.3396). In the low category (1-10 ticks) the difference between the proportion of cattle in the ISMM treated villages and control villages was also not statistically significant from day zero to day 140 (Figure 6.4 and Appendix 9 [Table 9.1b]). The difference was only significant at day 84 post treatment (95% CI = from -0.2571 to -0.0447; d.f. = 1; p-value= 0.005; χ^2 = 7.8772) when the proportion was higher in the control villages. In the medium category the difference between the proportion of cattle in the ISMM villages and control villages was statistically significant only at day 140 (95% CI = from -0.2222, -0.0445; d.f. = 1; p-value= 0.0035; χ^2 = 8.5051) post treatment (Figure 6.4 and Appendix 9 [Table 9.1c]). After day zero in the high category it was significant only at day 28 (95% CI = from 0.0057 to 0.0771; d.f. = 1; p-value= 0.0155; χ^2 = 5.8623) post treatment (Figure 6.4 and Appendix 9 [Table 9.1d]).

Pour-on treated villages compared with control villages

From day 28 to day 140 the proportion of animals in the zero category (no ticks) in the pour-on treated villages were high and statistically significant ($p < 0.05$) from the proportion of animals that were in the control villages (Figure 6.5 and Appendix 9 [Table 9.2a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the pour-on treated villages and control villages were statistically significant ($p < 0.05$) at day 28, day 84 and day 112 (Figure 6.5 and Appendix 10 [Table 9.2b). The proportion was high in the pour-on villages.

In the medium category (11-50 ticks) the proportion of animals was high and statistically significant ($p < 0.001$) in the control villages compared with the pour-on villages from day 28 to day 140 (Figure 6.5 and Appendix 9 [Table 9.2c]). In the

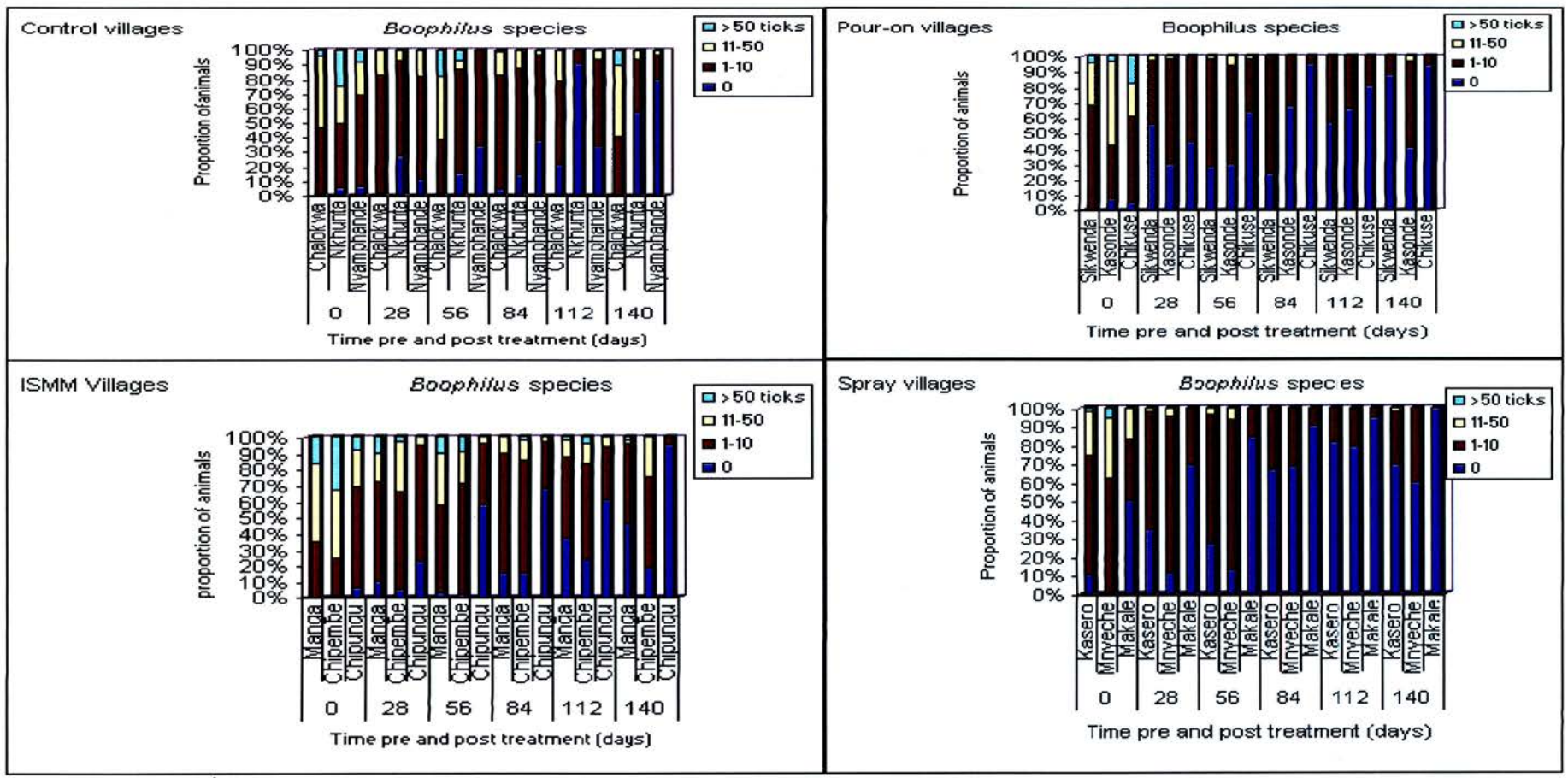
high category (> 50 ticks) the proportions of animals were high and statistically significant ($p < 0.05$) in the control villages compared with the pour-on villages at day 56 and day 140 (Figure 6.5 and Appendix 9 [Table 9.2d]).

Restricted application (RA) treated villages compared with control villages

From day 28 to day 140 proportion of animals in the zero category (no ticks) in the RA treated villages were high and statistically significant ($p < 0.05$) from the proportion of animals that were in the control villages (Figure 6.5 and Appendix 9 [Table 9.3a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the spray treated villages and control villages were statistically significant ($p < 0.05$) also from day 56 to day 140 (Figure 6.5 and Appendix 9 [Table 9.3b]). At day 28 the proportion was high in the spray villages and between day 84 and 112 it was high in the control villages.

In the medium category (11-50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the RA villages from day 28 to day 140 (Figure 6.5 and Appendix 9 [Table 9.3c]). In the high category (> 50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the spray villages at day 56 and day 140 (Figure 6.5 and Appendix 9 [Table 9.3d]).

Figure 6. 5 Proportion of animals with tick burden (*Boophilus* ticks) in the categories 0= zero ticks, category 1 = 1-10 ticks, category 2= 11-50 ticks, category 3 = > 50 ticks under various treatment regimes. Spray = restricted application of insecticide (RA)



6.3.8.9.3. Proportion of animals in each tick (*Rhipicephalus* species) burden category under various treatment regimes.

Isometamidium chloride (ISMM) treated villages compared with control villages

The proportion of animals in the zero category (no ticks) in the ISMM treated villages were statistically significant only at day 28, day 112 and 140 ($p < 0.05$). The proportions were high in the ISMM treated villages (Figure 6.6 and Appendix 10 [Table 10.1a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the ISMM treated villages and control villages was statistically significant only at day 28 and day 140 when the proportion were high in the control villages (Figure 6.6 and Appendix 10 [Table 10.1b]).

In the medium category the difference between the proportion of cattle in the ISMM villages and control villages was statistically significant ($p < 0.05$) from day 28 to day 84 (Figure 6.6 and Appendix 10 [Table 10.1c]). The proportion was high in the control villages. In the high category (> 50 ticks) there was no difference ($p > 0.05$) in the proportion of animals in the ISMM treated villages compared with the proportion of animals in the control villages (Figure 6.6 and Appendix 10 [Table 10.1d])

Pour-on treated villages compared with control villages

From day 28 to day 140 the proportion of animals in the zero category (no ticks) in the pour-on treated villages were high and statistically significant ($p < 0.05$) from the proportion of animals that were in the villages that were not treated (Figure 6.6 and Appendix 10 [Table 10.2a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the pour-on treated villages and control villages were statistically significant ($p < 0.05$) at day 28, day 84, day 112 and day 140 (Figure 6.6 and Appendix 10 [Table 10.2b]). The proportion was high in the pour-on villages at day 28 and day 84. At day 112 and day 140 the proportion was high in the control group.

In the medium category (11-50 ticks) the proportion of animals was high and statistically significant ($p < 0.001$) in the control villages compared with the pour-on villages from day 28 to day 84 (Figure 6.6 and Appendix 10 [Table 10.2c]). In the high category (> 50 ticks) the proportions of animals were high and statistically

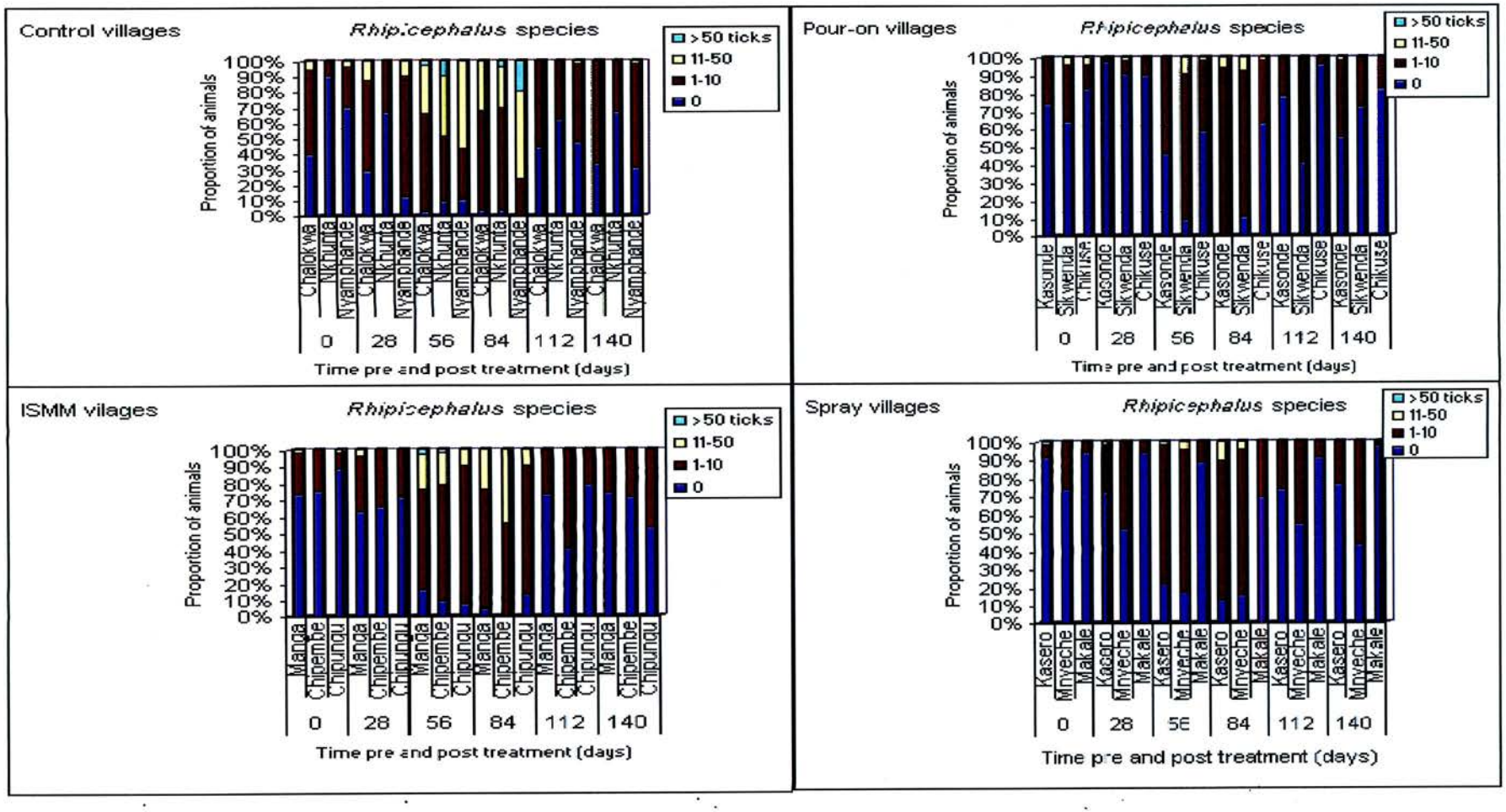
significant ($p < 0.05$) in the control villages compared with the pour-on villages at day 56 and day 84 (Figure 6.6 and Appendix 10 [Table 10.1d]).

Restricted application (RA) Spray treated villages compared with control villages

From day 28 to day 140 proportion of animals in the zero category (no ticks) in the RA treated villages were high and statistically significant ($p < 0.05$) from the proportion of animals that were in the villages that were not treated (Figure 6.6 and Appendix 10 [Table 10.3a]). In the low category (1-10 ticks) the difference between the proportion of cattle in the RA treated villages and control villages were statistically significant ($p < 0.05$) at day 28 and from day 84 to day 140 (Figure 6.6 and Appendix 10 [Table 10.3b]). At day 28 the proportion was high in the spray villages and between day 84 and 140 it was high in the control villages.

In the medium category (11-50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the RA villages from day 28 to day 84 (Figure 6.6 and Appendix 10 [Table 10.3c]). In the high category (> 50 ticks) the proportion of animals was high and statistically significant ($p < 0.05$) in the control villages compared with the RA villages at day 56 and day 84 (Figure 6.6 and Appendix 10 [Table 10.3d]).

Figure 6. 6 Proportion of animals with tick burden (*Rhicipephalus* ticks) in the categories 0= zero ticks, category 1 = 1-10 ticks, category 2= 11-50 ticks, category 3 = > 50 ticks under various treatment regimes. Spray = Restricted application of insecticide (RA)



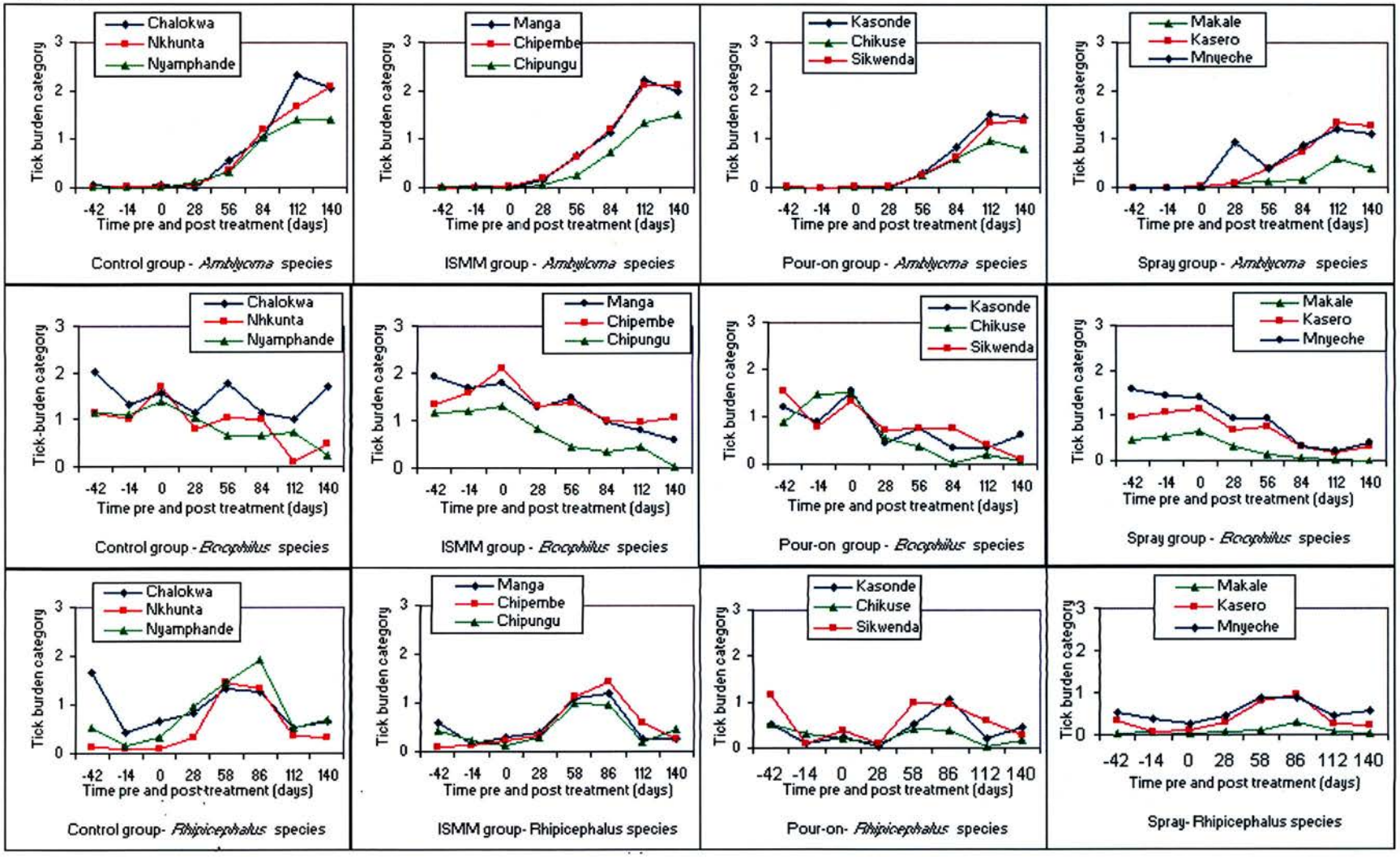
6.3.8.9.4. Monthly tick burden on cattle under various treatment regimes

The results showed that very few *Amblyomma* ticks were seen on animals between day -42 (May/June) and day 28 (July). The number started increasing from day 56 and peak numbers occurred between day 112 (November) and day 140 (December). During the peak period this species was seen more in villages that lie on the southern part of the study area (Chalokwa, Kasero, Nkhunta, Manga, Chipembe, Kasonde, Sikwenda and Mnyeche) and less in villages on the northern part (Makale, Chikuse, Chipungu and Nyamphande) (Figure 6.7).

Boophilus species was observed throughout the study period with slightly higher numbers seen between May (day -42) and July (day 0). After treatment (day 0), the number of ticks seen on animals in the spray and pour-on treatment villages were less than in the control and isometamidium chloride villages (Figure 6.7).

Rhipicephalus species were present throughout the area during the study period from May (day -42) to December (Day 140). Before treatment, this species was found more in villages (Chalokwa, Manga, Sikwenda) that lie on the southern part of the study area. A few ticks were found on animals in the villages (Makale) on the northern part of the area (Figure 6.7). Two peaks were seen in the area, one between May and June (day-42 and day -14) and a second one between day 28 (August) and day 84 (October). After treatment started there were less ticks observed on cattle that were in the villages under pour-on and spray treatments (Figure 6.7).

Figure 6. 7 Tick burden on cattle under various treatment regimes. Categories 0= zero ticks, category 1 = 1-10 ticks, category 2= 11-50 ticks, category 3 = > 50 ticks.



6.3.8.10. Persistence of *Theileria* parasites infection in cattle.

Persistence of *Theileria* parasites was only looked at in the control groups that did not receive any insecticide treatment. The persistence of *Theileria* parasites in cattle that were positive was determined by analysing each individual animal that became positive in the three control villages during the longitudinal study in Petauke District. Analysis of the results of the *Theileria* parasites detected by microscopic examination showed that out of the 87 cattle that were infected in the three control villages, 79.3% (69/87) were detected with parasites on one visit only, 14.9% (13/87) on two visits and 5.7% (5/87) on three visits during the study (Table 6.12a). By PCR 82% (91/111) cattle were detected with the parasites on one visit only, 17.1% (19/111) on two visits and 0.9% (1/111) on three visits during the study (Table 6.12b). No individual animal was found positive for the *Theileria* parasites on more than three visits.

Table 6. 12a Number of times individual animals found positive by Microscopy

	Villages				
Number of visits found positive	Chalokwa	Nkhunta	Nyamphande	Total number of animals positive	Percentage positive (%)
One	26	27	16	69	79.3
Two	8	5	0	13	14.9
Three	5	0	0	5	5.7
	39	**32**	**16**	**87**	

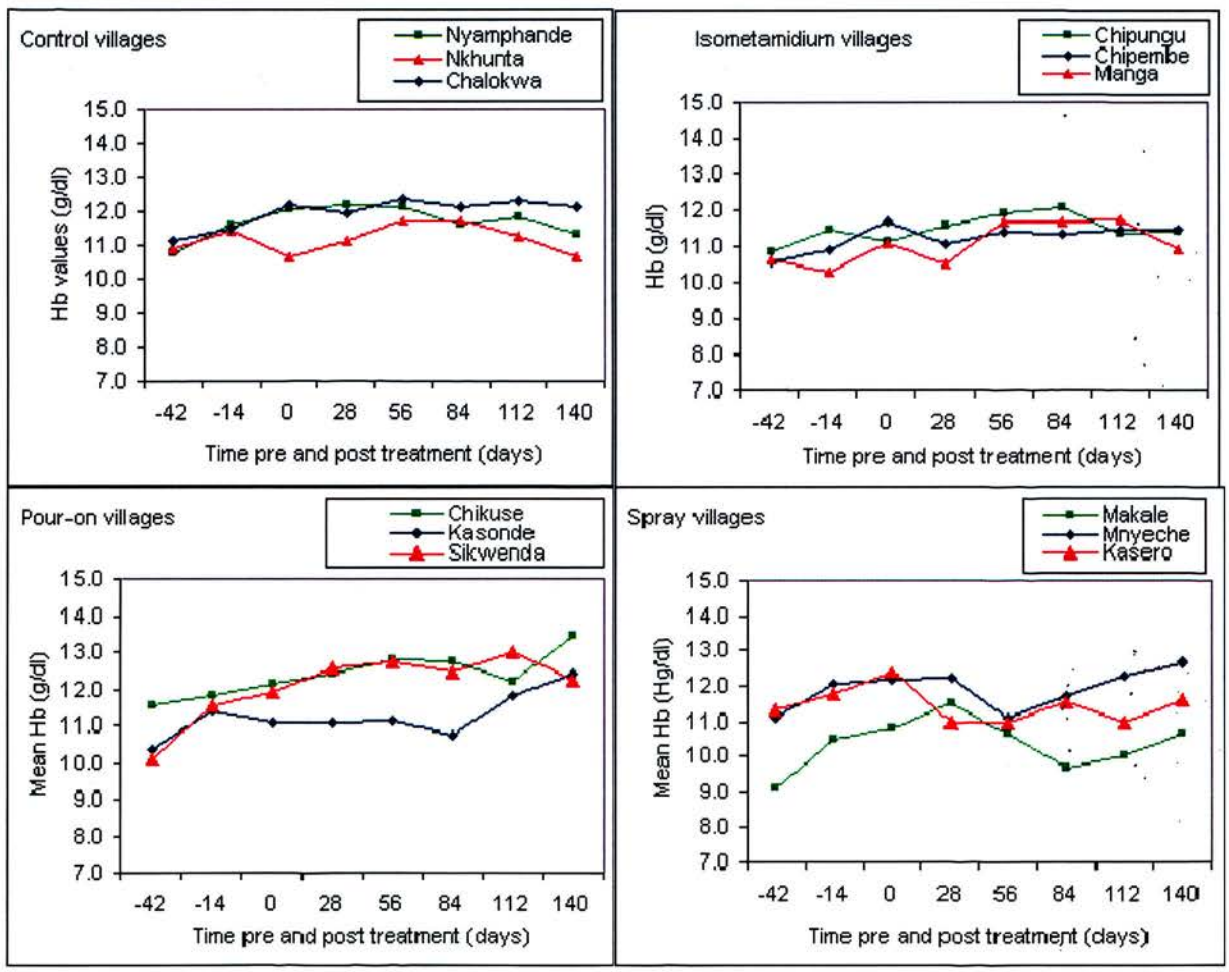
Table 6. 12b Number of times individual animals found positive by PCR

	Villages				
Number of visits found positive	Chalokwa	Nkhunta	Nyamphande	Total number of animals positive	Percentage positive (%)
One	41	25	25	91	82
Two	11	4	4	19	17.1
Three	0	0	1	1	0.9
	52	**29**	**30**	**111**	

6.3.9. Haemoglobin concentrations of cattle under various treatment regimes in the 12 villages of Petauke.

The normal range of haemoglobin value in healthy cattle is 8-15g/dl (Schalm *et al.*, 1980). The haemoglobin concentration of cattle in all the treatment regime groups increased after they were treated with diminazene aceturate at day -42 and day -14 prior to the first intervention (day 0). In the control and isometamidium chloride treated cattle the mean haemoglobin values started decreasing from day 84 and day 112 respectively post first intervention (Figure 6.8 and Appendix 11) while in the pour-on and restricted application treated animals the mean values continued to increase from day 84. The lowest mean haemoglobin values were at Makale that had the highest number of trypanosome infected cattle during the study. The mean haemoglobin value at Makale rose from 9.13g/dl (baseline survey; day -42 prior to first intervention) to 10.6g/dl (day 140 post first intervention) and the difference of 1.47g/dl was statistically significant (95%CI: 0.686 to 2.25; d.f. = 129; t = 1.98).

Figure 6. 8 Haemoglobin values for cattle in the 12 villages of Petauke District.



6.3.9.1. Treatment effect on haemoglobin concentration of cattle in the isometamidium chloride (ISMM) treated villages.

The monthly mean haemoglobin concentration in the isometamidium chloride treated and control cattle are shown in Figure 6.8 and Appendix 11 (Table 11.1a). There was little difference between the control and ISMM treated cattle. From day zero the difference was statistically significant only at day 28 and day 56. The mean haemoglobin at day 28 was lower in the treated cattle, 11.07g/dl compared to 11.69g/dl in the control (untreated) cattle (95% CI = -1.139 to -0.408; d.f. = 345; t = -4.16; p= <0.001). At day 56 it was 11.57g/dl in the ISMM treated cattle compared to 12.06g/dl in the control cattle (95% CI = -0.895 to -0.097; d.f. = 308; t = -2.44; p = 0.015)

6.3.9.2. Treatment effect on haemoglobin concentration of cattle in the Pour-on treated villages.

There was little difference between the control and pour-on treated cattle from day zero to day 84 (Figure 6.8 and Appendix 11[Table 11.1b]). The difference was only statistically significant at day 114 and day 140. The mean haemoglobin concentration in the pour-on treated cattle at day 112 was higher, 12.34g/dl compared to 11.75g/dl in the control (untreated) cattle (95% CI = 0.191 to 0.988; d.f. = 304; t-value = 2.91; p-value= <0.004). At day 140 it was also higher in the pour-on treated cattle, 12.68g/dl compared to 11.52g/dl in the control cattle (95% CI = 0.705 to 1.601; d.f. = 313; t= 5.06; p = < 0.001).

6.3.9.3. Treatment effect on haemoglobin concentration of cattle in the Restricted application of insecticide (RA) treated villages.

The mean monthly haemoglobin concentration values for the control and spray treated cattle are shown in Figure 6.8 and Appendix 11 (Table 11.1c). The results show that from day zero, there was no difference between the control and spray treated cattle at day 28 and day 140. The mean haemoglobin at day 56 was lower in

the spray treated cattle, 10.8g/dl compared to 12.06g/dl in the control (untreated) cattle (95% CI = from -1.669 to -0.865; d.f. = 359; *p*-value= <0.001; t-value = -6.2). At day 84 it was also lower in the spray treated cattle, 11.04g/dl compared to 11.75g/dl in the control cattle (95% CI = -1.207 to -0.391; d.f. = 351; *p*-value= <0.001; t-value = -3.85). At day 112 the mean haemoglobin concentration remained lower in spray treated cattle, 11.59g/dl compared to 11.75g/dl in the control cattle (95% CI = -0.919 to -0.149; d.f. = 331; *p*-value= 0.007; t-value = -2.73). However the mean haemoglobin concentration in the RA continued rising while that of the control groups was going down and by day 140 the RA treated cattle had values higher (11.59g/dl) than for the control (11.52g/dl), although they were not statistically different (95%CI: -0.420 to 0.550; d.f. = 314; t = 0.26; $p = 0.792$).

6.3.10. Body condition of cattle under various treatment regimes

There was no significant difference in the body condition of the cattle in all the various treatment groups (Figure 6.9 and Appendix 12). In all the treatment groups the proportion of animals in the fat category went down as the study progressed. In the lean category of all the groups the proportion also went down (Figure 6.9 and Appendix 12).

6.3.10.1. Effect of isometamidium chloride (ISMM) on the body condition of cattle.

From day zero to day 140 post treatment there was not much difference between the proportion of cattle in the control and ISMM treated groups in the fat category (Figure 6.9 and Appendix 11 [Table 12.1a]). In the medium category the difference between the proportion of cattle in the control and ISMM treated groups was statistically significant only at day 28 ($p < 0.05$) post treatment (Figure 6.9 and Appendix 11 [Table 12.1b]). In the lean category it was also statistically significant only at day 28 ($p < 0.05$) post treatment (Figure 6.9 and Appendix 11 [Table 12.1c]).

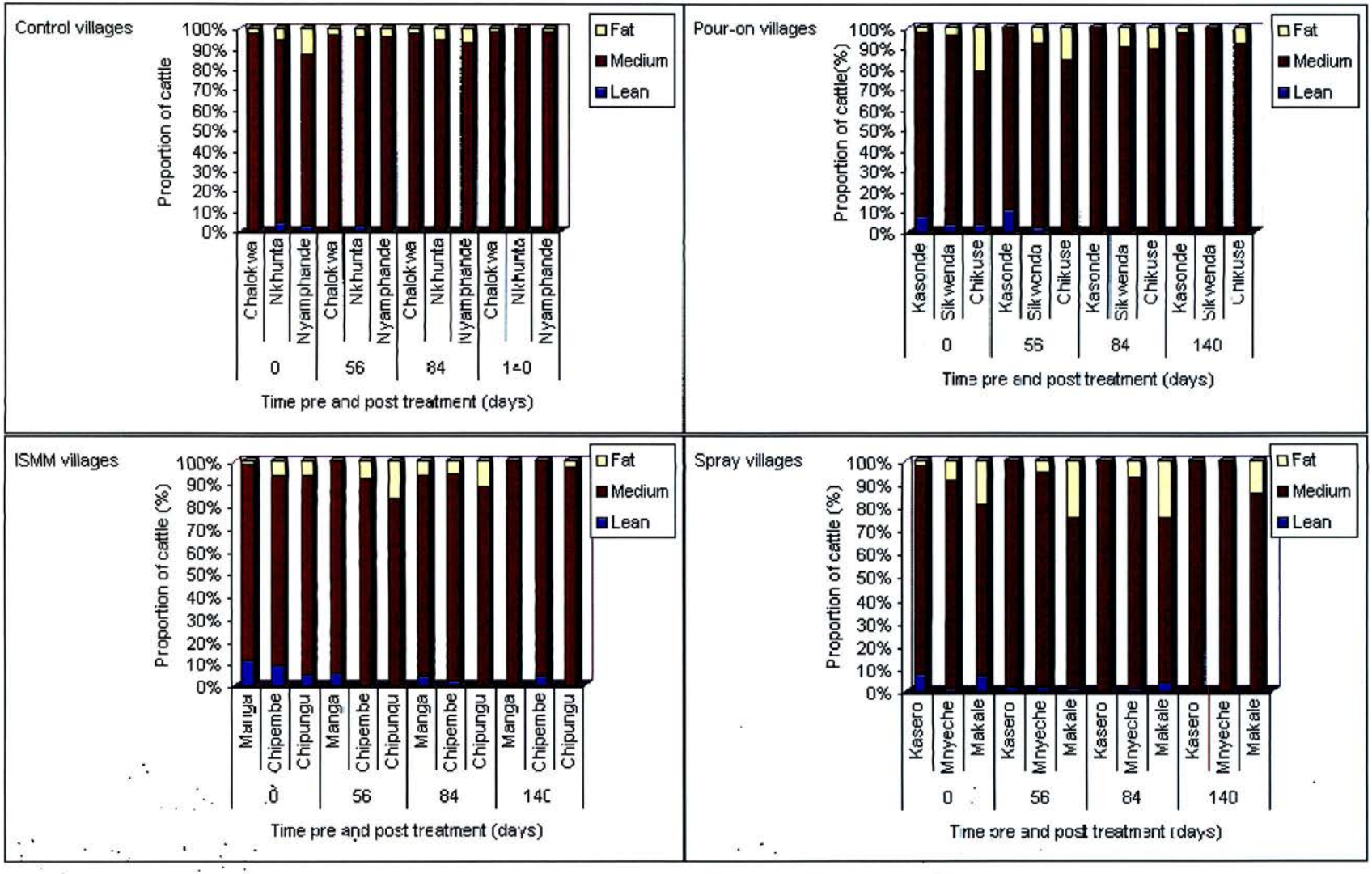
6.3.10.2. Effect of pour-on treatment on the body condition of cattle.

The difference between the proportion of cattle in the control and pour-on treated groups was not statistically significant ($p > 0.05$) from day zero to day 140 in the fat category (Figure 6.9 and Appendix 11 [Table 12.2a]). In the medium category the difference between the proportion of cattle in the control and pour-on treated groups was statistically significant only at day 56 ($p < 0.05$) post treatment (Figure 6.9 and Appendix 11 [Table 12.2b]). In the lean category it only approached significant only at day 28 ($p < 0.05$) post treatment (Figure 6.9 and Appendix 11 [Table 12.2c]).

6.3.10.3. Effect of restricted application of insecticide (RA) cattle on the body condition of cattle.

In the fat category the difference between the proportion of cattle in the control and RA treated groups was statistically significant only on day 28, day 56 and day 112 ($p < 0.05$) post treatment (Figure 6.9 and Appendix 11 [Table 12.3a]). In the medium category the difference between the proportion of cattle in the control and RA treated groups was statistically significant also on day 28, day 56 and day 112 ($p < 0.05$) post treatment (Figure 6.9 and Appendix 11 [Table 12.3b]). In the medium category the proportions were high in the control group. In the lean category there was no difference ($p > 0.05$) in the proportion between the control and the RA treated groups from day zero to day 140 post treatment (Figure 6.9 and Appendix 12 [Table 12.3c]).

Figure 6.9 Effect of treatment on body condition of cattle in the 12 villages of Petauke. Proportion of cattle in each category per treatment villages.



6.4. Discussion

6.4.1. Trypanosomiasis

After cattle were treated with diminazene aceturate on day –42 and day -14 before the first intervention (day 0), there were apparent very few cattle found with trypanosomes infection by either microscopic examination of the Giemsa stained blood thick and thin smears or PCR analysis. Only 2 cases were revealed by PCR at day -14, after the first diminazene aceturate treatment. Very few infections were detected in the area in the subsequent months and this was mainly at Makale village that lies closer to the fly front of the Luangwa valley (Tables 6.3a and 6.3b). The proximity of Makale village to the South Luangwa National Park in the Luangwa valley may have influenced trypanosomiasis incidence detected during the study period. The park has abundant game that act as reservoirs for trypanosomes. Unfortunately, the low re-infection rate in other villages made it difficult to compare infection rates in the different treatment groups to determine which group performed better.

The mean haemoglobin value was lowest at Makale where most of the infected cattle were diagnosed among the 12 villages in the study area. Although the mean haemoglobin value at Makale was lower than in the other villages it continued rising (from 9.13g/dl to 10.6g/dl), an indication of the impact of the restricted application of insecticide on the animals in this village.

6.4.2. Tick borne diseases

The incidence of *Theileria parva* parasites as revealed by PCR analysis shows that in the pour-on and restricted application of the insecticide treated villages it was higher during the first few months and low towards the end of the study. This was not the case in the control and isometamidium chloride treated villages where it was higher towards the end of the trial (Table 6.4a and Figure 6.2a). This suggests that pour-on and restricted application of the insecticide did have some effect on ticks on the animals that were in these villages. It also showed that cumulative number of positive animals for *Theileria parva* continued to rise until the end of the study in the

control villages and those villages where animals were treated with isometamidium chloride. In the villages where cattle were treated with insecticide in the form of pour-on or spray, the cumulative total number of cases increased initially but after the third application the number remained constant (Appendix 6a and 6b). The results by microscopic examination showed high incidence for animals with *Theileria* species two months (day 56) after the first intervention in all the villages (Table 6.2b and Figure 6.1b). Cumulative number of positive animals for *Theileria species* remained high in the two control villages apart from Nyamphande village. It also remained high at Mnyeche (spray village) and Chikuse (pour-on village) until the end of the study (Appendix 6a and 6b). Microscopy results showed more cases than PCR analysis at day 56. This was because PCR used (p104 gene) was specific and detected only *Theileria parva* whereas the microscopy examination detected both *Theileria parva* and other *Theileria species* found in the area of study. The other *Theileria species* found in the area are *Theileria mutans*, *Theileria velifera* and *Theileria taurotragi* (Makala *et al.*, 2003). Unlike *T. Parva*, the piroplasm stage of *T. mutans* divides in erythrocytes of cattle (Young *et al*, 1988) and this may make it easier to be detected by microscopic examination of the thin and thick blood smears stained with Giemsa stain. *T. mutans* and *Theileria velifera* are transmitted by *Amblyomma* species of ticks which were also seen increasing in numbers from day 56 (Figure 6.4 and Appendix 8)

Observation made on the persistence of infection in cattle during this study showed that there was an on-off (intermittent) presence of the *Theileria* parasites in cattle diagnosed by both microscopy and PCR analysis (Table 6.12a and 6.12b). Cattle that were at one time positive for the parasites were not showing parasiteamia in the subsequent months suggesting that the species of parasites we were looking at probably did not induce a long term carrier state that is defined as a persistent of tick-transmission infection (Oura, *et al.,* 2004)

The results of the PCR analysis showed that more cattle remained un-infected with *Theileria parva* in the villages that were treated with the restricted application of the insecticides. When the isometamidium chloride treated villages were compared with the control villages the increase in the survival proportional was only 3.3% (95% CI:

from -11.7% to 18%). When the Pour-on treated villages were compared with the control villages the increase in the survival proportional was only 2% (95% CI: from -12.6% to 16%). When the control villages were compared with the villages that were treated with the restricted application of the insecticides villages the increase in the survival proportional was 17.5% (95% CI: from 3.2% to 31%). The results of microscopy examination of the thick and thin blood smear for *Theileria* species showed similar pattern apart from cattle in the villages that were treated with the restricted application of the insecticides. Compared with the control villages the increase in the survival proportion was 5% (95% CI: -9.5% to 18.7%) in the isometamidium chloride treated villages, 4% with a (95% CI: from -11.9% to 19.5%) in the pour on villages and only 1% (95% CI: from -13.6% to 14.7%) in the villages that were treated with the restricted application of the insecticides. PCR results gave a figure of 15% because it was detecting animals that were positive for only *Theileria parva* whereas Microscopy examination detected animals that were positive for all the species available in the area. The results by microscopy examination doubled compared to PCR analysis of the proportion of animals that survived infection in the isometamidium chloride and pour-on treated villages. This is because PCR analysis is much sensitive compared to microscopy examination (Picozzi *et al*, 2002). The PCR technique used in this study was able to detect twice the number of *Theileria* parasites that was detected by microscopy (Chapter 5, Section 5.3.1.2.)

Cattle that were infected with other tick-borne parasites (*Anaplasma* species and *Babesia* species) were in low numbers throughout the study period and this made it difficult to do any analysis on this data.

It was observed that most of the animals that died of theileriosis during the course of the study were in the age group of less than 4 years (Table 6.9 and Figure 6.3). Older animals that have been exposed to high levels of ticks are known to develop resistance to ticks (Perry *et al.*, 1984; Walker *et al.*, 1987; Latif and Pegram, 1992; Mwangi *et al.*, 1998) and become less susceptible to theileriosis as they develop resistance to the disease (Bakheit and Latif 2002).

Ticks were seen more on animals in the villages that lie on the southern part of Petauke District. *Amblyomma* ticks were rarely seen on animals between day -42 (May/June) prior to the intervention and day 28 (July) after the first intervention (Figure 6.6). The number started increasing from day 56. *Amblyomma* tick numbers were higher on animals that were in control and isometamidium chloride treated villages compared to those that were in the pour-on and restricted application of insecticides treated villages, an indication that the later two treatments were performing better than the former two (Figure 6.7). *Boophilus* species were observed throughout the study period and after the first intervention (day 0), the number of ticks seen on animals in the spray and pour-on treatment villages were less than in the control and isometamidium chloride villages (Figure 6.7). *Rhipicephalus* species were present throughout the area during the study period from May (day -42) to December (Day 140). Two peaks were seen in the area, one between May and June (day-42 and day -14) and a second one between day 54 (September) and day 84 (October). After the first intervention during the second peak of *Rhipicephalus* species few ticks were observed on cattle that were in the villages under pour-on and spray treatments compared with those that were in the control and isometamidium chloride treated villages. The Control Villages had the highest number of ticks throughout the study period (Figure 6.7). Similar seasonality pattern in *Amblyomma, Boophilus* and *Rhipicephalus* tick burden have been observed in Eastern province by Pegram *et al.* (1986) and Berkvens *et al.* (1998). In this study Amblyomma ticks increased with the on- set of the raining season (Table 6.1 and Figure 6.1)

Proportion of animals that were in the tick-burden categories 0 (Zero ticks) and 1 (1-10 ticks) increased and those in categories 2 (11-49 ticks) and 3 (>50 ticks) decreased after the first intervention in the villages that were treated with pour-on and of restricted application of insecticide compared to the proportion of animals that were in the isometamidium chloride and control villages (Figures 6.4, 6.5 and 6.6). Pour-on and restricted application of insecticide have been known to reduce tick-burden and tsetse flies on cattle (Vale, *et al.*, 1999; Mekonnen, *et al.*, 2000; Torr *et al*, 2007).

6.4.3. Effect of treatment on haemoglobin concentration of cattle in the 12 villages of Petauke.

The results showed after treating the animals with diminazene aceturate at day -42 and day -14 prior to the first intervention, there was an improvement in haemoglobin concentration in all the treatment groups. Towards the end of the trial at about day 84 after the first intervention there was a decrease in haemoglobin concentration values in the animals in the control and the isometamidium treatment groups. The pour-on and the restricted spray groups showed an increase in haemoglobin concentration towards the end of the trial an indication that the spray and pour-on groups were doing better than the other two groups (Figure 6.9). Pour-on treated group did perform better than spray group.

6.4.4. Effect of different treatments on body condition of cattle in the 12 villages of Petauke.

The results from this study showed that there was no significant effect of the isometamidium chloride, pour-on and restricted application of insecticide on the body condition of the cattle. These results were consistent with an investigation on the evaluation of a novel method using deltamethrin for controlling bovine trypanosomiasis in Uganda (Brownlow, 2007). In our study the proportion of cattle in all treatment groups in the fat category reduced as the study progressed probably as the result of the seasonal effect (Table 6.1 and Figure 6.1). Rains result in abundance of good pasture for grazing and as the rains come to an end in May the amount of the grass also reduces and by August the area is virtually void of good grazing grass.

7. CHAPTER SEVEN

7. GENERAL DISCUSSION

7.1. Overview

Tsetse- and tick-borne diseases continue to be important in the Eastern Province of Zambia where they have adversely affected the livelihoods of the rural population. In spite of the importance of tsetse- and tick-borne diseases, the control of these diseases is no longer the responsibility of the Zambian government and most control activities have now been shifted to private sector. The Zambian Government now only monitor and regulate activities that are contracted to private companies. The studies presented in this thesis investigated the epidemiology of trypanosomiasis and the impact of the disease on the rural population in Mambwe District, Eastern Province of Zambia. Mambwe District lies partly in the Great Rift Valley system of Eastern and Southern Africa, and partly in the Eastern Plateau. The thesis further evaluated the use of restricted application of synthetic pyrethroids insecticide onto cattle to control tsetse- and tick-borne diseases on the plateau in Petauke District.

Currently the control of tsetse- and tick-borne diseases depends on vector control through the use of insecticide-treated targets and insecticide-treated cattle e.g. pour-on in case of tsetse flies and acaricides in plunge dipping tanks or sprayers in case of ticks. Chemotherapeutic and prophylactic methods are also used extensively against trypanosomes in sub-Saharan African countries (Geerts and Holmes, 1998; Delespaux *et al*, 2002; Van den Bossche, *et al*, 2000; Geerts *et al*, 2001). However acaricides and prophylactic drugs are expensive and most of the farmers in the rural affected areas are very poor and cannot afford these services. Chapter three of this thesis examined the livelihood of the people in Mambwe District. The use of synthetic pyrethroids to target both ticks and tsetse has potential advantages, for instance reduced cost (Eisler *et al*, 2003). For maximum effect of integrated control, information on the epidemiology of these diseases will give an indication as to when optimum strategies for vector control will be achieved. Chapters four, five and six of this thesis looked at this aspect.

7.2. Trypanosomiasis in livestock in Mambwe District and its effect on livelihoods of immigrant population

The plateau of the Eastern Province of Zambia is an area of high agriculture potential and 8% (190,671) of the national cattle herd is located in this part of the country (DVLD, Annual Report 2004). On the plateau of the Eastern Province the cattle population has been increasing over the past decade from 5 head/km^2 in 1997 to 10/km^2 in 2004 and much of the land has been cleared for crop production (Van den Bossche and Staak, 1997; Van den Bossche *et al*, 2004). In the past two decades, this has forced some famers to emigrate from the plateau into the valley of Mambwe District where the soils are fertile (Mambwe District, First quarterly Report, 2005). The fertile soils of the valley provide farmers with good pasture for their animals and rich alluvial soils for crop production. The farmers have continued moving into the valley despite of their knowledge of the existence of trypanosomiasis.

One of the old methods used to control tsetse was by bush clearing i.e. cutting down habitat of tsetse. Intensive land-use by farmers where the land is cleared of vegetation for crop production is an indirect method of tsetse control as the area is void of tsetse habitat. The impact of habitant modification by either deliberate control interventions (old method of tsetse control) or intensification of land-use by human is of huge importance in tsetse control and epidemiology of trypanosomiasis (Jordan, 1986). The gradual decrease in density of tsetse and wild hosts in settled areas, which become unsuitable for the fly results in the increase in number of cattle (Van den Bossche, 2001). In the valley where the study was conducted there are few people hence less habitat modification and high tsetse density (chapter 3).

The study conducted in an area of the valley heavily infested with tsetse flies, has demonstrated that most of the households in this area survive on about one dollar per day (Chapter 3, Section 3.3.2.5), with over 80% of the households depending on cotton farming for their income. People living in this area are poor with average income per household per annum of about ZMK1,849,131 (US$377.85). The construction of houses is generally of poor quality and over 60% are made out of thatched roofs and muddy walls. This is a clear indication of poverty. The mean

number of cattle kept in the valley (1 ox/household) and the low level of milk produced per cow (1 litre/cow/day) demonstrated the negative effect trypanosomiasis has on livestock production. In the sub-Saharan Africa milk production in tsetse infested area is about 4 times less than milk produced in areas that are tsetse-free (Kristjanson, *et al*, 1999).

The rate at which the farmers and their animals are moving from the plateau into the valley, where the soils are prone to erosion (Chapter 3; Mambwe District, First quarterly Report, 2005), threatens to exceed the land carrying capacity. The land carrying capacity should be determined and monitored if environmental damage such as land degradation and soil erosion and loss of fertility is to be avoided. It could be useful if the Department of Resettlement, Department of Veterinary and Livestock Development, traditional Chiefs and all the stakeholders come together and plan on how best to settle people who are coming into the area.

This study revealed that there is inadequate veterinary service in Mambwe District in the area where this research was carried out. There was no local Veterinary Officer or Veterinary Assistants or shops where the farmers could buy veterinary drugs, as a result farmers had to travel long distances to neighbouring districts to purchase veterinary drugs. Travelling to other districts was hampered by lack of public transport. When required, Veterinary Officers came from the Provincial Headquarters in Chipata District located about 120km from the study area.

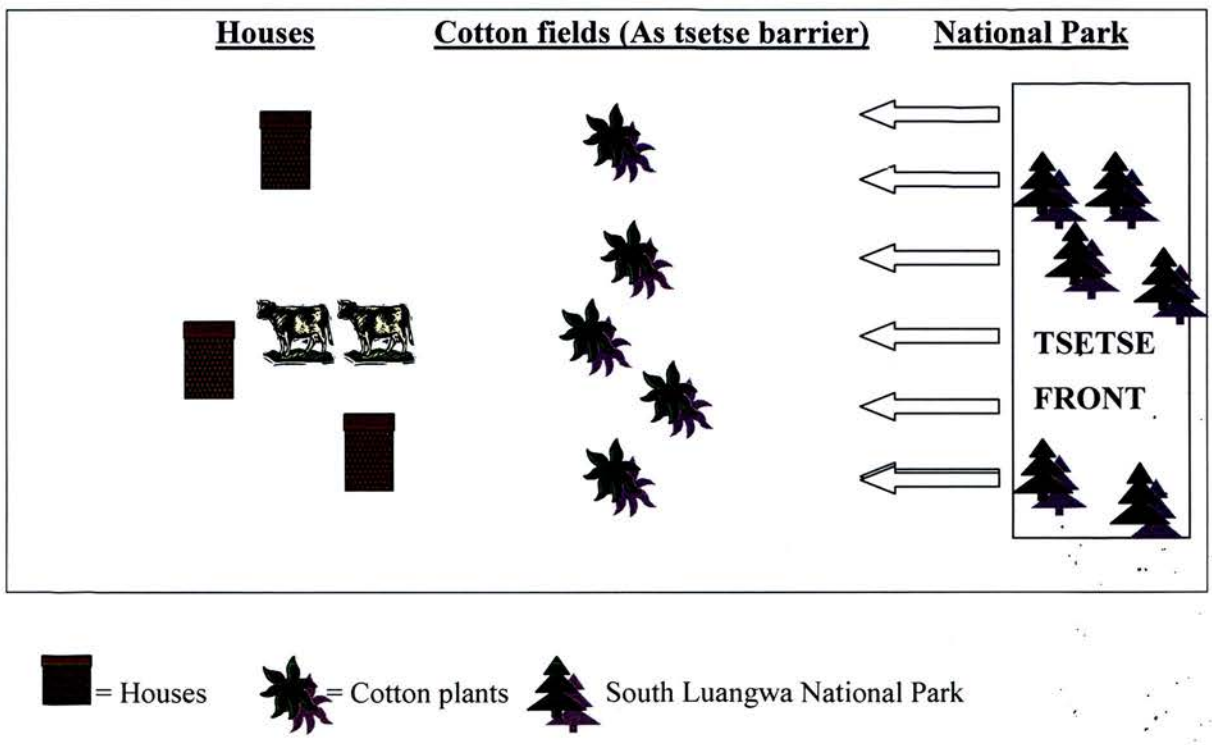
Synthetic pyrethroids are effective in controlling tsetse when used on targets (Thomson, 1987; Vale, 1993a). Targets impregnated with synthetic pyrethroids can be used as a barrier to tsetse. Tsetse barriers using impregnated insecticide treated targets have been used in many infested areas (Vale, *et al*, 1988a). In Zambia tsetse impregnated insecticide treated black screens have been used successfully to prevent tsetse re-invasion into tsetse controlled areas of Siavonga District in Southern Province and Chaiwa area in Lower Zambezi in Lusaka. Insecticide treated cattle have also been used as a barrier (Van den Bossche and Mudenge, 1999).

Strategic spatial arrangement of cotton crops between the tsetse invasion front and the homesteads where cattle are kept in Mambwe District might be investigated as a means to reduce tsetse-borne disease (Figure 7.1). Farmers in Mambwe District

already spray their cotton plants with deltamethrin (chapter 3). In this arrangement, the pyrethroid-treated crops might act as a barrier between the tsetse invasion front and the cattle, in a similar manner to the use of targets and insecticide treated cattle, and might help reduce the incidence of bovine trypanosomiasis. The similarity between the cotton crops and using targets and insecticide-treated cattle as a tsetse barrier is that they all use deltamthrin to kill tsetse. Both targets and insecticide-treated cattle are baited technologies whereas cotton crop is not. Cotton crop is not bait technology and will only kill tsetse when they fly through it. Tsetse flies make many short flights (Hargrove, 2000) and are likely to rest on cotton crop when they fly through the fields. Tsetse will be attracted by the cattle that will be on the other side of the fields (Figure 7.1).

This method might be used alone or possibly used in integration with other methods such as the pour-on, restricted application of deltamethrin on cattle and use of trypanocides in sick animals.

Figure 7. 1 Cotton fields acting as a tsetse barrier



7.3. Prevalence of trypanosomiasis in Mambwe District, Eastern Province, Zambia

The cross-sectional study conducted in the highly tsetse-infested area extending from the base of the valley to the plateau in Mambwe District showed that altitude, combination of livestock kept at household level, sex and age of animals all have a significant impact on the prevalence of trypanosomiasis.

The prevalence was high in the low altitude area because of its proximate to the South Luangwa National Park. The National Park is the source of tsetse flies. Prevalence was high in males than females observations that have been made also by Rowlands *et al.* (1993, 2001), Dolan (1998). The prevalence of trypanosomiasis in particular livestock species also depended on the combinations kept within households. Small ruminants were more likely to be infected if cattle were present. In households keeping any combination of livestock, cattle had the highest infection. Cattle in herds are known to produce larger odour plumes than pigs or small ruminants and this makes tsetse flies more attracted to them (Torr *et al.* 2006). In households that were keeping just one animal species the trypanosomiasis prevalence was highest in cattle (28.4% [95%CI: 23.7-33.5]) followed by pigs (21.5% [95%CI: 13.9-31.8]), sheep (18.2% [95%CI: 5.1-47.7]) and goats (9.2% [95%CI: 6.8-12.4]). The high trypanosomiasis prevalence in small ruminants may limit the impact of control within the cattle industry as in the absence of treatment these small ruminants may continue to act as a reservoir for trypanosomes within this area. In Mambwe District, no significant efforts are made, either by the farmers or veterinary officials, to control trypanosomiasis in small ruminants and pigs (chapter 3). Controlling of the trypanosomes in small ruminants and pigs may contribute greatly to trypanosomiasis control in Mambwe District.

In all the species of animals, the effect of age on overall prevalence of trypanosomiasis showed higher prevalences in older animals than young ones. In cattle the prevalence was lowest in the age category of 1-12 months and in the other livestock species in the age category of 1-6 months (chapter 4, Tables 4.5 and 4.6 and Figures 4.2 and 4.3). Similar results have been observed by Trail *et al.* (1994), Rowlands *et al.* (2001) and Simukoko *et al.* (2007). This is because tsetse flies are least attracted by the odour of calves (Torr and Mangwiro, 2000; Torr et al, 2006).

The other reason could be that the calves are kept at homestead when the others go for grazing (personal observation).

The major trypanosome species circulating in cattle in the area was *T. congolense* savannah type (74%), *T. vivax* (23%) and *T. brucei* (2%). In cattle *T. congolense* is considered the most pathogenic trypanosome (Naylor, 1971; Valli et al, 1978; Valli et al, 1980; Bengaly *et al.*, 2002a; Bengaly *et al.*, 2002b; Masumu, *et al*, 2006). *Trypanosoma congolense* prevalence was higher in older animals than younger ones while *T. vivax* prevalence was higher in younger animals. These patterns of *T. vivax* and *T. congolense* infections with age have also been demonstrated by Trail *et al.* (1994). Individuals that were infected with trypanosomes had lower haemoglobin concentration values (in cattle = 8.53 g/dl) than those that were negative (10.2 g/dl), an observation that could play a vital role in the study of epidemiology of trypanosomiasis in the area. It was also observed that haemoglobin concentration values for the individual positive animals were lower in animals that were infected with *T. congolense* than other species, an observation that has also been reported in Nigeria (Omeke, 1994).

Similar patterns of trypanosomes species prevalence in other livestock species in Mambwe District were observed but *T. simiae* was only seen in pigs. Ten out of 37 pigs found positive were infected with *T. simiae*. We found two of the infected pigs that we followed up three days after sampling dead demonstrating the pathogenicity of the parasite that is virulent in this species of animals. This study showed 5 out of 29 (17.2%) positive pigs were infected with *T. vivax* based on PCR analysis. Microscopic examination of the thick and thin smears did not reveal any *T. vivax* infection in pigs. The presence of *T. vivax* in pigs has also been demonstrated in Kenya by Ng'ayo *et al.* (2005) and in Cameroon (Simo, *et al.*, 2006). In pigs that were infected with *T. simiae* the haemoglobin concentration (13.2g/dl) did not differ from those that were negative (13.2g/dl) but the other trypanosome species did lower the haemoglobin concentration (chapter 4, Table 4.21). This is because pigs infected with *T. simiae* die within a short time of infection (Finelle, P. 1983; Boyt, 1986).

Currently the control of trypanosomiasis in Mambwe District is only by administering of trypanocides to livestock. Despite extensive operations on the

Eastern Plateau, e.g. Katete and Petauke Districts, no control method targeting the vector has been used in the valley. Survival analysis showed that 25% of cattle that were reported to have been treated with isometamidium chloride by farmers or local veterinary officials became infected by day 73 post treatment, 50% became infected by day 122 and 75% by day 153 (chapter 4). The median survival time (time until half the cattle become infected) was 122 days (95%CI: 100 to 129). The finding of this work is in agreement with the protection period in areas that are heavily infested with tsetse where prophylactic period is about 3 months (Boyt, 1986). Although the results indicate that isometamidium chloride is effective in the area, there is an indication of treatment problem as 18.5% (5/27) of the animals that were treated with isometamidium chloride were positive for trypanosomes within 1 month of reported treatment and 24.4% (76/311) within 2 months of reported treatment (chapter 4). The problem could be due to incorrect drug dilution or dosage (chapter 4). The other reasons could be famers gave wrong dates the animals were treated or the drugs were given incorrectly e.g. subcutaneously. The break through infections also could not be ruled out. In the treated animals the species that re-infected the livestock was *T. congolense* (chapter 4). Trypanocides are used at a large scale in this district by the farmers who have little knowledge on how to dilute or administer them. If the usage of these drugs is not controlled it may lead to drug resistance (Geerts *et al*, 2001). Resistance to trypanocidal drugs has been reported in the neighbouring districts of Katete and Petauke (Mubanga and Sinyangwe, 1997).

7.4. Tsetse- and Tick-borne disease in Petauke District

7.4.1. Prevalence of trypanosomes (Baseline survey)

The baseline survey was conducted to collect baseline data on the prevalence of the *Anaplasma spp*, *Babesia spp*, *Theileria spp* and *Trypanosome species* in Petauke District where a trial of restricted application of insecticide trial was to be conducted. Cattle were sampled at 12 villages that had shown more than 5% of trypanosomiasis prevalence in the earlier surveys conducted in the area (Hopkins, *et al,* 1998; Machila *et al*., 2001; Sinyangwe *et al*, 2004).

The results of this study showed that more cattle were infected with trypanosomes in villages that are located on the northern part of the study area. The observations are consistent with the earlier studies conducted in the area (Machila *et al*, 2001). The villages that are on the northern part of the study area lie near the South Luangwa National Park that harbours both tsetse flies and abundant wildlife that may act as reservoirs for trypanosomes (Dillmann and Townsend, 1979). Only one village (Chalokwa) on the extreme southern part of the study area had high prevalence of trypanosomes. Chalokwa is close to the Mozambique boarder and on this part of Mozambique no tsetse control has taken place because of the civil unrest that occurred in the 1980s and late 1990s.

7.4.2. Mean haemoglobin value for animals positive for trypanosomes

The mean haemoglobin value of animals that tested positive for trypanosomes by microscopy (9.2 g/dl) was lower than that of the negative animals (10.7g/dl) for the baseline survey. The same was observed for the animals that were positive by PCR where the mean for the positive animals was 9.25 g/dl while it was 10.77 g/dl for the negative ones (tables 5.8 and 5.9 and figure 5.9a and 5.9b). This was statistically significant in both cases (t-test; $p = 0.001$). Trypanosomiasis is well been known to reduce haemoglobin concentration in animals and is one of the clinical signs that has been widely used in diagnosis of this disease (Sadun, *et al.*, 1973; Babatunde, *et al.*, 1982).

7.4.3 Prevalence of *Theileria* parasites (Baseline survey)

In contrast to the distribution of trypanosomes, many cattle infected with *Theileria* parasites were found in the villages that were located on the south-eastern part of the study area. The villages on the extreme western and northern part of the study area had low prevalence (chapter 5; Figure 5.2). The finding of this study is in agreement with Berkvens *et al* (1998) who observed that the spread of one of their vector *Rhipicephalus appendiculatus* was from the east to the west of Petauke District.

Theileria species found in the study area include *T. parva*, *T. mutans, T. velifera* and *T. taurotragi*. (Makala *et al,* 2003)

7.4.3. Mean haemoglobin value for animals positive for *Theileria* parasites

The haemoglobin concentration of cattle that were diagnosed positive for *Theileria* parasites by microscopy (10.00 g/dl) was statistically significant from cattle that were negative for the parasites (10.75 g/dl). The haemoglobin concentration of cattle that were diagnosed positive for *Theileria parva* by PCR (10.48 g/dl) was not statistically significant from cattle that were negative for the parasites (10.73 g/dl). PCR analysis used in this study only detected *Theileria parva* parasites. These results are consistent with other findings were anaemia was found not to be the main clinical syndrome in cattle infected with *T. parva* (Young *et al*, 1988). *Theileria mutans* causes anaemia because they divide and multiply in the red blood cells (Young *et al*, 1988). Microscopic examination detected but could not distinguish all the *Theileria* parasites including those that cause anaemia like *T. mutans* (Saidu, *et al.,* 1984).

7.4.4. Evaluation of restricted application of insecticide on cattle in Petauke District

The evaluation of restricted application (RA) of deltamethrin, a synthetic pyrethroid applied to only the legs, belly and ears of cattle to control tsetse- and tick-borne diseases, was conducted as a longitudinal study in Petauke District. The effect of the RA on trypanosomiasis and tick-borne diseases, cattle haemoglobin concentration, body condition and was compared with pour-on, isometamidium chloride and control groups.

7.4.5. Effect of restricted application of deltamethrin on *Theileria* parasites.

Based on PCR analysis more cattle remained un-infected with *Theileria parva* in the villages that were treated with the RA of the deltamethrin. When compared with the control villages, the increase in the survival proportional (proportion that remained

uninfected) for the RA of deltamethrin was 17.5% (95% CI: from 3.2% to 31%). When the control villages were compared with isometamidium chloride it was 3.3% (95% CI: from -11.7% to 18%) and when control was compared with pour-on the survival proportion was 2% (95% CI: from -12.6% to 16%). This shows that the RA did have a significant effect on *Rhipicephalus appendiculatus* the vector of *Theileria parva* the parasite that cause East Coast fever.

Based on examination of the thick and thin blood smear for *Theileria* species more cattle remained infected with *Theileria species* in the villages that were treated with the restricted application of the deltamethrin. When compared with the control villages, the increase in the survival proportional (proportion that remained uninfected) for the RA of insecticide was only 1% (95% CI: from -13.6% to 14.7%). When the control villages were compared with isometamidium chloride treated villages it was 5% (95% CI: -9.5% to 18.7%) and when control was compared with pour-on treated villages the survival proportion (proportion that remained uninfected) was 4% with a (95% CI: from -11.9% to 19.5%). Microscopic examination revealed that cattle in RA villages did not do better than the other two treatments. The proportional of cattle that did not get infected was lower than for isometamidium chloride and pour-on villages.

An explanation for a high proportion of cattle that survived infection of *Theileria parva* as determined by PCR analysis may be due to the fact that *Rhipicephalus appendiculatus* the vector of these parasites are found on the ears which were thoroughly sprayed with the deltamethrin. One of the main predilection feeding site for *Rhipicephalus appendiculatus* are the ears; the common name for this species of ticks being the brown ear tick. It could also be that most of the cattle in the study area are survivors of calfhood acute infection with *T.parva* and thus have high degree of immunity to East Coast fever. Microscopic examination was also diagnosing other *Theileria* parasites that included the *T. mutans* transmitted by other ticks like *Amblyomma variegatum* (Paling *et al*, 1981; Saidu *et al*., 1984) that attach to other parts of the body like the dewlap (personal observation). During this study some of *Amblyomma* ticks were seen on the neck region of cattle. With the restricted application of deltamethrins, these anatomical areas were not sprayed. This could

possibly also explain why microscopic examination diagnosed more cattle with *Theileria* parasites than PCR analysis at day 56 after the first intervention. This was the same time *Ambylomma* ticks were just picking up in numbers in the study area.

PCR analysis for *Theileria parva* showed cumulative number of positive animals continued to rise until the end of the study in the control villages and those villages where animals were treated with isometamidium chloride. In the villages where cattle were treated with deltamethrin in the form of pour-on or restricted application, the cumulative total number of cases increased initially but after the third application the number remained constant. This is likely to represent successful interruption of transmission (chapter 6, Figure 6.1a and 61b.).

7.4.6. Effect of restricted application of insecticide on *Anaplasma* and *Babesia*.

The number of cattle infected with *Anaplasma* and *Babesia* parasites was very low throughout the study period. These two parasites seem not to cause much problems in this area. All the cattle in the study area are Angoni breed (Zebu). These results are in consistency with observations made by Young *et al*, (1988). Anaplasma and Babesia cause few problems in local breeds in areas of endemic stability (Young *et al,* 1987d; Young *et al*, 1988).

7.4.7. Effect of restricted application of insecticide on trypanosomes.

The low trypanosome prevalence in cattle after they were treated with diminazene aceturate made it difficult to determine the effect the restricted application of insecticide had on cattle. Throughout the study the prevalence was low in all the villages that were under different treatment regimes including controls. It is unfortunate that the study did not cover much of the rainy season part of the year. This was because bridges on the roads leading to most of the villages were in bad conditions and in rainy season made access to the sampling sites difficulty. In Zambia the monthly incidence of trypanosomiasis is high during rainy season (November to April) and highest during the end of the rains when tsetse population reach peak densities (Connor, 1993). This study was conducted from May to

December. Trypanosomes that were circulating in cattle in this area were cleared by the diminazine aceturate that was administered to the cattle at day -42 and day -14 prior to the first intervention. There was a great improvement in the animal haemoglobin concentration values after the administration of this trypanocide, indicating that there was a significant effect on the parasites that were causing anaemia. Trypanosomes are well known to produce anaemia in livestock and is one of sign that is used in trypanosomiasis diagnosis (Sadun, *et al*, 1973; Babatunde, *et al*, 1982).

7.4.8. Effect of restricted application of deltamethrin on haemoglobin concentration.

Blood is an important medium in assessing the health status of animals (Ariyibi, *et al*, 2002). This study looked at the haemoglobin concentration values as a means of assessing the health of cattle that were treated with either isometamidium chloride or insecticide in the form of either pour-on or restriction of insecticide application on cattle to control tsetse and tick-borne diseases. The effect of the restricted application of deltamethrin on cattle did show a significant increase in the mean haemoglobin value only after day 84 post first intervention. This was also observed in cattle in the villages that were treated with pour-on. During the same period the haemoglobin concentration values of the animals in the control and isometamidium chloride treated villages started to drop (Chapter 6, Figure 6.8 and Appendix 11).

7.4.9. Effect of restricted application of deltamethrin on body condition.

There was no significant effect of the restricted application of deltamethrin on the body condition of the cattle compared with those in the control group. The same observation was made in cattle that were in the villages that were treated with isometamidium chloride and pour-on.

7.4.10. Conclusion and recommendations

The findings of this work give an opportunity to the Government policy makers and all the stakeholders who are involved in tsetse control to come up with appropriate disease control strategy for this area and areas that have similar vector ecology and disease epidemiological pattern. Based on our findings in Mambwe District, if isometamidium chloride is to be used in a form of prophylaxis treatment against trypanosomiasis it is recommended that it is administered at three to four months interval. Frequent use of this drug may lead to resistance. Geerts and Holmes (1998) suggested that the best way to delay the development of drug resistance is to reduce selection pressure on parasite populations. This can be done by reducing the treatment frequency, using correct dose and reducing the number of animals treated (Geerts and Holmes, 1998). Number of animals treated may be reduced by only treating valuable or susceptible cattle like working oxen, pregnant and lactating animals. An opportunity also exists to create tsetse barrier by strategic resettlement of immigrant farmers who grow cotton in such a way that their cotton fields are placed between the villages and the tsetse front so that they act as barriers against infestation.

From the longitudinal study investigation it may be concluded that the restricted application of deltamethrin prevented *Theileria parva* infection (Chapter 6, Table 6.4a) but failed to prevent the infection due to other *Theileria* parasites. At day 56 and day 112 after the first intervention there was a rise in the number of *Theileria* parasites (Chapter 6, Table 6.4b). East Coast fever (ECF) caused by *Theileria parva* is the most important tick-borne disease in Zambia (Nambota *et al*, 1994). Restricted application of deltamethrin to cattle must be recommended in Eastern Province to control ECF and trypanosomiasis because it has several advantages over the conventional methods of dipping the whole animal in a dipping tank (Chizyuka *et al.*, 1986; Luguru *et al.*, 1993) or spray race and pour-ons (Van den Bossche *et al.*, 2004). Poor management of acaricides in traditional sector is one of the disadvantage of using diptanks. Low acaricide concentrations in most of the dip-tanks is as a result of lack of knowledge of dip-tanks capacity that leads to wrong charging and incorrect replenishment (Luguru, *et al.*, 1985). Furthermore water to replenish the dip-tanks is

usually a problem in most of the rural areas affected by these tsetse- and tick-borne diseases and these facilities are prone to vandalism (personal observation). The benefits the farmers can get from using the restricted application of deltamethrin onto cattle include; increase efficiency, cost of insecticide reduced by 40%, reduce threats to non-target organism (Torr, *et al.*, 2007). Furthermore the method can be used at the same time to control ticks, tsetse flies and other biting flies (Bauer, *et al.*, 1992). The finding of this work has proved that the restricted application of the insecticide to only the belly, legs and ears does not remove all the ticks completely from the body of the cattle thereby providing a moderate tick challenge that is important for maintaining of the endemic stability of the tick-borne diseases (Eisler *et al.*, 2003).

Finally the finding of this study proved that microscopic examination of thick and thin smears cannot be used on its own in epidemiological surveys when accurate diagnosis of species of parasites is of interest. For example in Mambwe District cross-sectional survey, *T. simiae* could not be easily indentified from *T. vivax or T. congolense* by microscopic examination, whereas with PCR analysis it was very easy to identify. This study has underlined the value of supplementing microscopy with molecular methods. Microscopic examination must therefore be used only in combination with other technique as it is also known to be a low sensitive parasitological diagnostic tool (Picozzi, *et al.*, 2002). The advantages of microscopic examination are that it can be used in the field and the results are available within a short time.

7.5. Future work

1. Evaluation of restricted application (RA) of insecticide in an area where trypanosomiasis prevalence is high in Zambia. The area must be large enough not to allow mixing of cattle under various treatment regimes under the study. The area must also have a high cattle density and accessible through out the year. This will allow the study to include the rainy season part of the year when monthly trypanosomiasis incidence is high. In Zambia the dry season is from May to October and the rainy season is from November to April. Modality of

carrying out the study in the rainy season ought to be put in place before carrying out the next study.

2. Follow up studies on immigration of people and their cattle into Mambwe District. During our study there were few cattle in the area and most of the settlements were concentrated along the main road. It will be useful to know how this has changed since we did our last survey in 2005. It will also be useful to know if veterinary services have improved in the area as a result of the increase in the number of animals coming from the plateau. The area was denied veterinary services in the past because of the low number of animals in the area (personal observation).

3. Compare the virulence of different strains of *T. simiae* circulating in pigs in Mambwe District, Luangwa Valley.

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9. APPENDICES

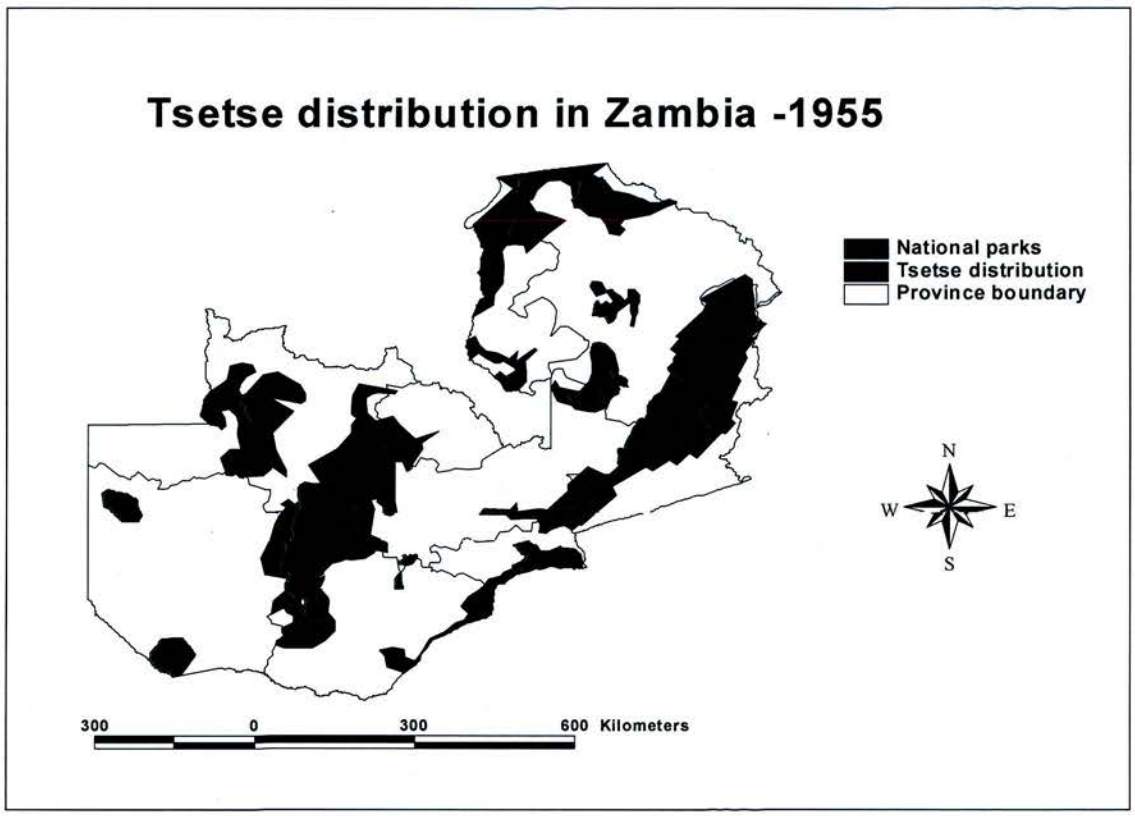
APPENDIX 1

Table 1. Cases of Human African Trypanosomiasis (HAT) from 1925 to 2004

Year	Cases	Year	Cases	Year	Cases	Year	Cases
1925	3*	**1946**	100	**1967**	110*	**1988**	-
1926	5*	**1947**	101	**1968**	77	**1989**	-
1927	22*	**1948**	60	**1969**	77*	**1990**	-
1928	6*	**1949**	73	**1970**	127*	**1991**	-
1929	0*	**1950**	169	**1971**	194	**1992**	-
1930	6*	**1951**	120	**1972**	395	**1993**	-
1931	4*	**1952**	38	**1973**	387*	**1994**	-
1932	12*	**1953**	38	**1974**	54	**1995**	-
1933	11*	**1954**	41	**1975**	91	**1996**	-
1934	13*	**1955**	30	**1976**	79	**1997**	-
1935	160*	**1956**	26	**1977**	137	**1998**	-
1936	28*	**1957**	20	**1978**	187	**1999**	**104****
1937	34*	**1958**	69*	**1979**	142	**2000**	**9****
1938	94*	**1959**	100	**1980**	58	**2001**	**6****
1939	30	**1960**	93*	**1981**	181*	**2002**	**43****
1940	52	**1961**	103*	**1982**	292	**2003**	**219****
1941	43	**1962**	81*	**1983**	183*	**2004**	**35****
1942	42	**1963**	108*	**1984**	-		
1943	83	**1964**	155*	**1985**	19		
1944	98	**1965**	99*	**1986**	-		
1945	112	**1966**	128*	**1987**	-		

Cases of human sleeping sickness in Zambia from 1925 to 2004. Source: C. Evison and K.D.S Kathuria, 1982, * = WHO offices, Lusaka . ** = Ministry of Health dataBase, Zambia. - = data not available.

APPENDIX 2



Map of Zambia showing tsetse distribution as in 1955. Source: C. Evison and K.D.S Kathuria, 1982

APPENDIX 3

HUMAN AND AGRICULTURE SURVEY QUESTIONNAIRE: ANIMAL HEALTH STUDY PROJECT, EASTERN PROVINCE ZAMBIA 2005

This section must be filled in before questionnaire

Date:..... **Enumerator:**.....

District:..... **Village:**.....

Chiefdom:..... **Latitude:**.....

Vet amp:..... **Longitude:**.....

Altitude:.....

DATUM: ARC 1960/.....

1. **Household**

Name of the Respondent.....

Gender of the Respondent: **female/male**

Name of Household head:.....

How many people slept in this household today?

Men	
Women	
Boys (≤ 15 years)	
Girls (≤ 15 years)	

a) How many people who are usually part of his household are not here today?

b) How many people who are not usually part of his household are here today?

	(a) Not here today	(b) Usually not here
Men		
Women		
Boys (≤ 15 years)		
Girls (≤ 15 years)		

2. Livestock

Do you own any livestock?YES/NO

How many of each type?

		Number	If None have you ever kept some in the past
Cattle	Yes/No		Yes/No
Goats	Yes/No		Yes/No
Pigs	Yes/No		Yes/No
Chickens	Yes/No		Yes/No
Guinea Fowls	Yes/No		Yes/No
Donkeys	Yes/No		Yes/No
Dogs	Yes/No		Yes/No

If cattle present, how many of each type?

Males	Number	Females	Number
Suckling male calves		Suckling female calves	
Weaned male <2yrs		Weaned female <2yrs	
Bulls (not castrated) ≥ 2yrs		Dry cows ≥ 2yrs	
Oxen (castrated) ≥ 2yrs		Milking cows ≥ 2yrs	

APPENDIX 4

HUMAN AND AGRICULTURE SURVEY QUESTIONNAIRE: ANIMAL HEALTH STUDY PROJECT, EASTERN PROVINCE ZAMBIA 2005

This section must be filled in before questionnaire

Date:..... **Enumerator:**.....

District:..... **Village:**.....

Chiefdom:..... **Latitude:**.....

Vet Camp:..... **Longitude:**.....

Altitude:.....

DATUM: ARC 1960/.....

1. Household

1.1 Name of the **Respondent**.....

1.2 Gender of the **Respondent**: **female/male**

1.3 Age of the respondent:.....

1.4 Relationship of the respondent to the head of the household:.....**Self**

1.5 Name of Household head:.....

1.6 Age of the head of the Household: .....

1.7 Highest level of Education attended by the Head of Household

Never went to school	
Primary school	
Secondary school	
University/College	

2. Household relocation

2.1 How long has this household been established here: .....

2.2 Where did the household come from?

Chiefdom	
Village	
District	

2.3 Why did the household move to this place?

1	
2	
3	

2.4 What is the main source of your income and how much do you get per annum?

Source	Amount (ZKW)

2.5 What type of a house do you leave in?

Muddy wall and grass thatched roof	
Muddy wall and iron/asbestos sheet roof	
Burnt bricks wall and grass thatched roof	
Burnt bricks wall and iron/ asbestos sheet roof	

2.6 What acreage does your Household/farm cover?

Total acreage of household/farm	
Acreage used for livestock	
Acreage used for crops	

2.7 Where do you get your drinking water from?

Piped water	
Borehole	
Well	
River	

3. Crops

3.1 What type of crops do you grow (**give Lima for each crop**)

Maize		Sorghum	
Cotton			
Tobacco			
Groundnuts			
Sunflower			
Millet			

3.2 If Cotton is grown.

How long does it take you to walk to the cotton field	
Name of insecticide used	
Source of insecticide	
Rate of application of insecticide	

3.3 In the rainy season do you shift to the crop fields from your homes?
YES/NO

3.4 Where do you sell your farm produce and how do you transport them?

Farm produce	Place where you sell farm produce	Means of transportation

3.5 Do you apply any fertilizer to your crops? **YES/NO**

3.6 Do you apply any manure to your crops? **YES/NO**

4.0 Mosquito control

4.1 Do you use mosquito bed nets? **YES/NO**

4.2 Do you use any drug? **YES/NO**

4.3 What are the names of the drugs?

1	
2	
3	
4	

4.4 How many malaria cases have you had this year? .....

4.5 How far is the nearest health centre? .....

5. Livestock

5.1 What type of livestock do you kept and if you don't what prevents you from keeping them?

Livestock	Yes/No	For how long have you kept them	Who takes care of the livestock	Where are they kept at night	Reasons for not keeping them
Cattle					
Goats					
Pigs					
Chicken					
Sheep					

5.2 Where did you get the livestock from?

Livestock	Bought	Inherited	Dowry	Others (1)	Others (2)
Cattle					
Goats					
Pigs					
Chicken					
Sheep					

5.1 What are the most important diseases affecting your livestock? (in the past one year)

	Livestock affected	Number affected	Number died	Main symptoms	Name of disease if known	Who diagnosed the disease	Who gave treatment	Season observed (dry or Wet or both)
Disease 1								
Disease 2								
Disease 3								
Disease 4								
Disease 5								

5.2 What drugs did you use?

Name of drug	Livestock given the drug	Number treated	Reason for treatment	Number died	Who gave the drug	Who chose the drug

5.3 How many livestock have died in the past two years?

Livestock affected	2004	2005	Total	Season died (dry or wet or both)
Cattle				
Goats				
Sheep				
Chickens				
Pigs				

5.4 Who gives your animals the drugs?

Farmer	
Other farmers	
Community Livestock Workers	
Local Veterinary Assistants	
Veterinary Officers	
Others	

5.5 How many calves were born in the last two years and how many have died?

	2003	2004
Born		
Died		

5.6 Where do you get the drugs from and do you pay for them?

	Source (Tick where applicable)	Do you pay **YES/NO**
"Briefcase" Vendors		
Community Livestock workers		
Local Vets		
Agro-vet shops		
Others (specify)		

5.7 If trypanocides, how do you prepare (dilute) the drugs for administration?

Name of the drugs	Dilution rate (How much drug/water)	Water source	Do you boil the water **YES/NO**	Dose rate (How much solution/animal)

5.8 Where do your animals graze during the rainy and dry seasons?

	Season	
	Dry	rainy
Around homesteads		
Plains		
Dambos		

5.9 Do you practice transhumance? **YES/NO**

5.10 Do you give your livestock feed supplement?

Livestock	Yes/No	Name of feed supplement
Cattle		
Goats		
Sheep		
Chickens		
Pigs		

5.11 How many litres of milk do you get per cow per day? (local measurement to be stated - cup or container or .....)

Livestock	litres
Cow 1	
Cow 2	
Cow 3	
Cow 4	

5.12 Do you sell milk from your cows? NO/YES

5.13 If yes.

In what form (Fresh or sour)	How much (ZKW) per Litre

5.14 Have you acquired or give away/sold livestock in the last one year?

Livestock	Yes/No	Number aquired	Number give away/sold
Cattle			
Goats			
Sheep			
Chickens			
Pigs			

5.15 Do you think flies are a problem in this area? **YES/NO**

5.16 Do you think ticks are a problem in this area? **YES/NO**

5.17 Do you use any spray, dip or pour-ons? **YES/NO**

5.18 Name of the product: .....

5.19 Do you handpick ticks from your livestock?

YES/NO

APPENDIX 5

5a. Isometamidium chloride dose rate manufacturer data sheet

1. Isometamidium chloride (Veridium®, CEVA SANTE ANIMALE).

Source: CEVA SANTE ANIMALE

PROPERTIES

Isometamidium, an aminophenanthridium compound, has trypanocidal action used in curative and preventive treatment of animal trypanosomiasis. Duration of preventive effect varies from 8 to 16 weeks according to the fly challenge.

INDICATIONS

Cattle (include zebu), buffaloes, sheep, goats, camels, equines, dogs: Curative and preventive treatment of trypanosomiasis.

ADMINISTRATION AND DOSAGE

Cattle, buffaloes, sheep, goats: Prepare the injectable solution by adding 100 ml of sterile water to each 1 g sachet (1% solution) or 1 g sachet into 50 ml of sterile water (2% solution). It is administered by deep intramuscular route in the neck muscle. See table below

WITHDRAWAL PERIODS

Meat: 1 month

Table 8.2 Isometamidium chloride dosage and administration

Body weight	**CURATIVE TREATMENT** (0.25 to 0.5 mg/kg)		**PREVATIVE TREATMENT** (0.5 to 1 mg/kg)	
	0.25 mg/kg	0.5 mg/kg		1 mg/kg
	1% SOLUTION		2% SOLUTION	
50 kg	1.25 ml	2.5 ml	1.25 ml	2.5 ml
100 kg	2.50 ml	5 ml	2.50 ml	5 ml
150 kg	3.75 ml	7.5 ml	3.75 ml	7.5 ml
200 kg	5 ml	10 ml	5 ml	10 ml
250 kg	6.25 ml	12.5 ml	6.25 ml	12.5 ml
300 kg	75 ml	15 ml	7.5 ml	15 ml
350 kg	8.75 ml	17.5 ml (div. 2 inj)	8.75 ml	17.5 ml (div. 2 inj)
400 kg	10 ml	20 ml (div. 2 inj)	10 ml	20 ml (div. 2 inj)
450 kg	11.25 ml	22.5 ml (div. 2 inj)	11.25 ml	22.5 ml (div. 2 inj)
500 kg	12.5 ml	25 ml (div. 2 inj)	12.5 ml	25 ml (div. 2 inj)
Number of 250 kg cattle treated with 1 g	16	8	8	4
Number of 250 kg cattle treated with 125 mg	2	1	1	0.5

5b Diminazene aceturate dose rate manufacturer data sheet

1 Diminazene aceturate (Veriben®, CEVA SANTE ANIMALE)

Source: CEVA SANTE ANIMALE leaflet

Administration and dosage

Deep intramuscular route.

The recommended dosage is 3.5 mg of diminazene aceturate per kg bodyweight in a single injection. Do not exceed total dose of 4 g of active ingredient in any animal.

Cattle, sheep, goats, horse: Prepare the injectable solutionby adding 15 ml of sterile water to each 2.36 g sachet. For the 23.6 g sachet it is necessary to add 150 ml of sterile water. Mix well water and powder before sucking the sterile solution into the syringe. Inject the prepared solution according to the following table:

Table 8.3 Diminazene aceturate dosage and administration

Body Weight	Volume of the reconstituted solution	As quantity of Diminazene aceturate
10 kg	2.30 ml	140 mg
50 kg	2.88 ml	175 mg
100 kg	5.8 ml	350 mg
200 kg	11.5 ml	700 mg
300 kg	17.3 ml	1050 mg
400 kg	23.0 ml	1400 mg
500 kg	28.8 ml	1750 mg

Dog: Reconstitute the injectable solution by adding 25 ml of sterile water to a 2.36 g sachet. Then, inject 1 ml of the reconstituded solution for 10 kg body weight (as 3.5 mg Diminizene aceturate per kg b.w).

PRECAUTIONS

In case of large volume to be injected, it is recommended to divide in two separate sites. Inject dogs strictly according to body mass. Overdosage or repeated dosing may cause central nervous symptoms in dogs.

WITHDRAWAL PERIODS

Meat: 21 days

STORAGE

Sealed sachets can be stored in a cool dry place below 30 °C. The reconstituted solution can be stored during 15 days in a fridge, protected from light and in a closed glass bottle.

2. Diminazene aceturate (Berenil®, Intervet)

Source: www.intervet.co.uk

Directions for Use: Use only as directed.

Cattle and horses: Dissolve a packet of 2,36 g Berenil® granules (1,05 g active) in 12,5 ml water (or a packet of 23,6 g in 125 ml) water for injection. When dissolved inject according to the following table, the dose for all animals being 3,5 mg active ingredient/kg body mass. It is recommended that a total dose of 4,0 g active Berenil® should not be exceeded even in the case of heavier animals.

N.B. The prepared solution is stable for 14 days if stored in a cool place, for 5 days at a temperature of ± 20 °C and for 3 days at 30 °C. At temperatures of 50 °C to 60 °C the solution is stable for ± 24 hours.

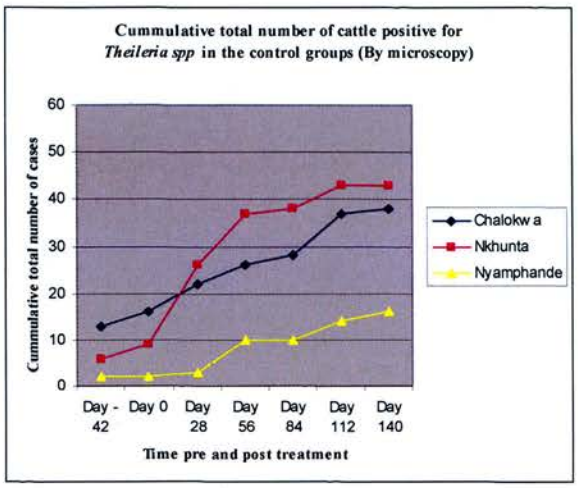
Table 8.3 Diminazene aceturate dosage and administration

Body mass (kg) Cattle and Horses	Volume of prepared solution for injection
10	0.5 ml
20	1.0 ml
100	5.0 ml
200	10.0 ml
500	25.0 ml

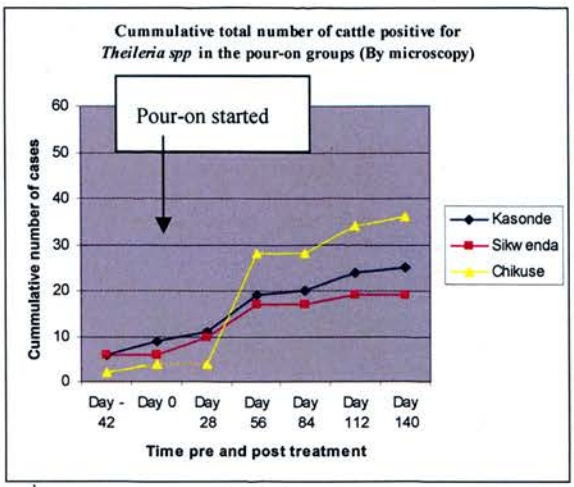
APPENDIX 6

Figure 6a Cumulative total number of cattle with *Theileria spp* (By microscopy)

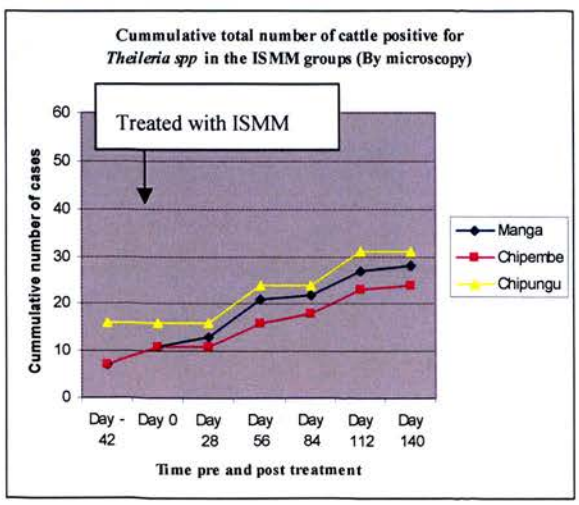
Graph a



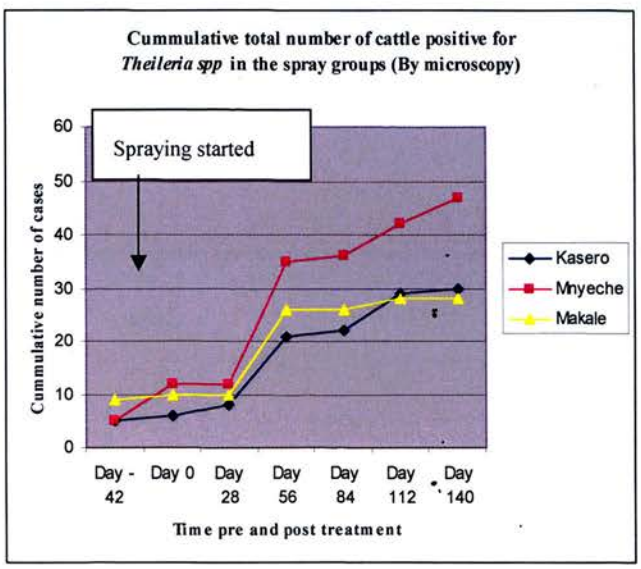
Graph c



Graph b



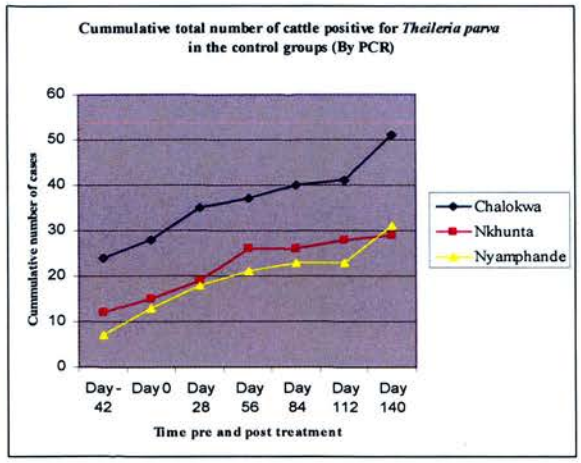
Graph d



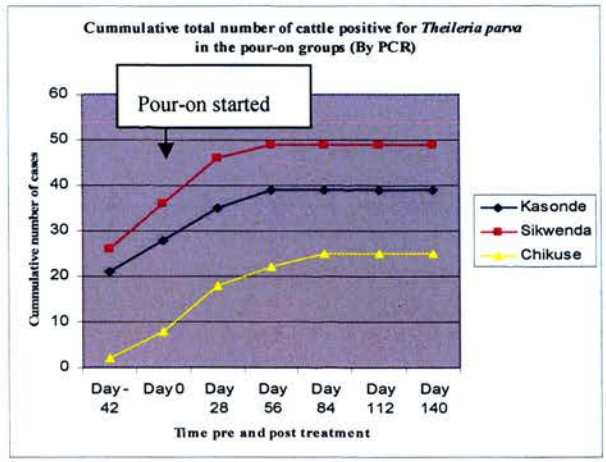
ISMM = Isometamidium chloride

Figure 6b. Cumulative total numbers of cattle with *Theileria parva* (By PCR amplification)

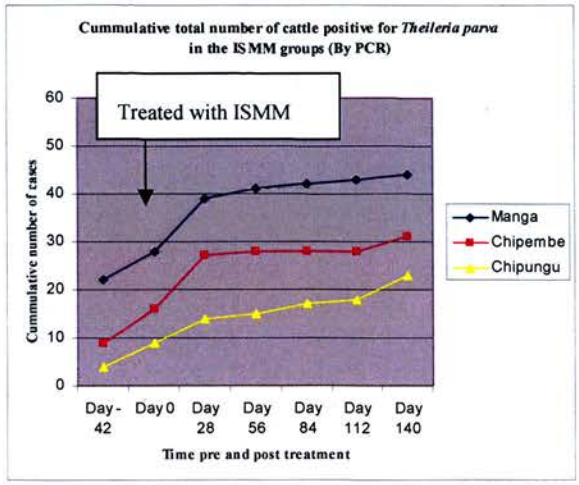
Graph a



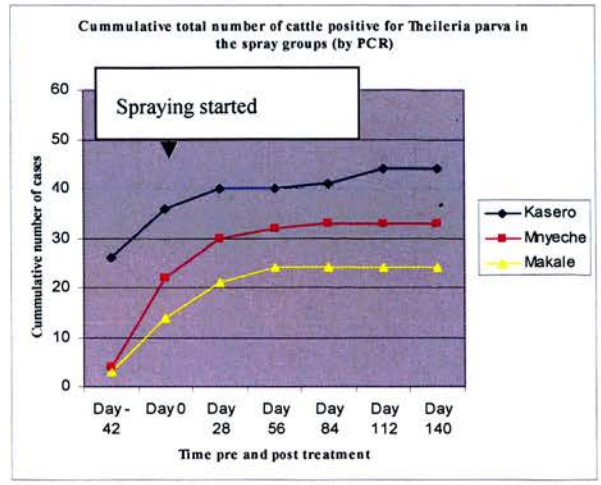
Graph c



Graph b



Graph d

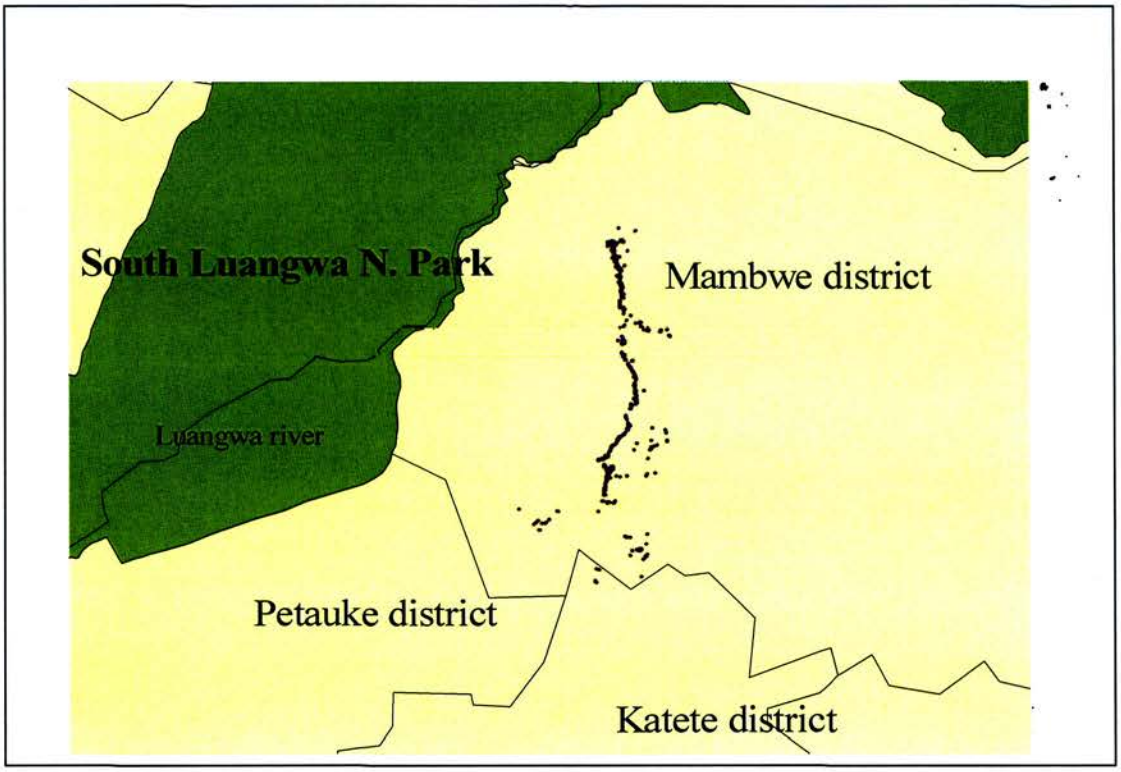


ISMM = Isometamidium chloride

APPENDIX 7

Appendix 7.1

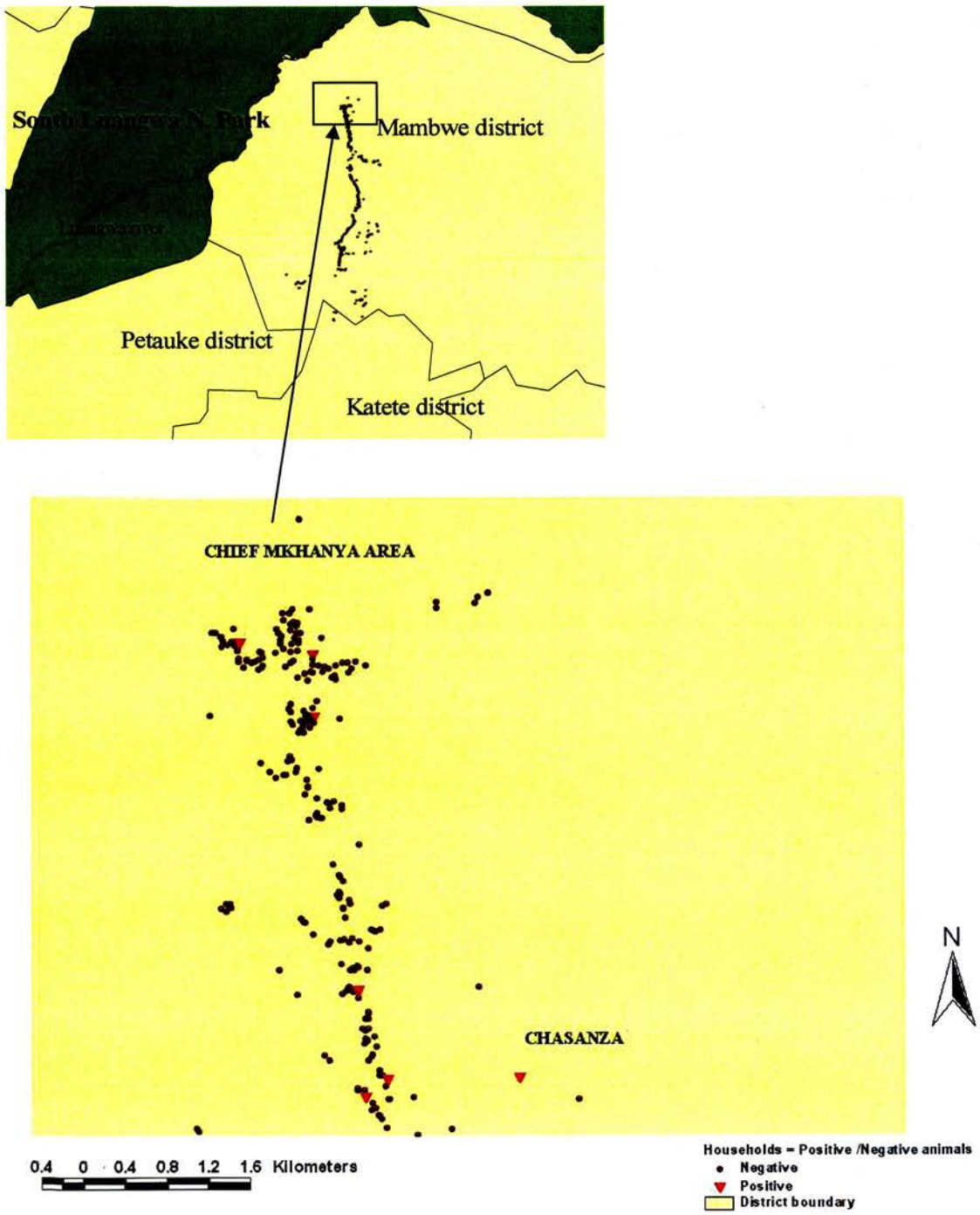
Map 7.1 Showing households in the study area in Mambwe district, Eastern province, Zambia. Households are concentrated alone the main road from the valley to the plateau.



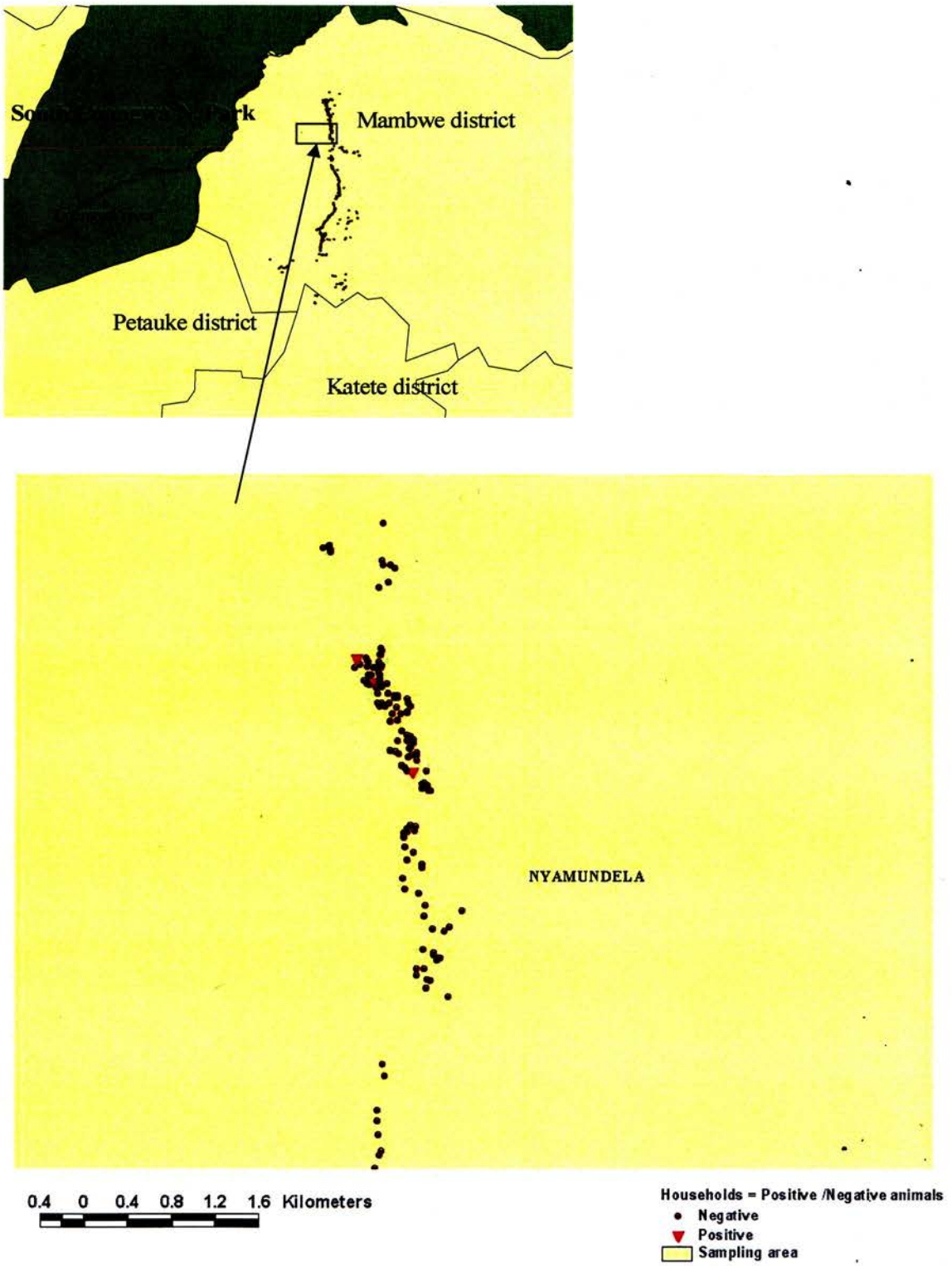
• = Households in Mambwe district from the valley to the plateau. To show the households clearly the area has been labelled from 1 to 6 and zoomed in (shown by a rectangle), in the following maps below. Maps 7.1a-7.1f show households with positive or negative animals. Maps 7.2a-7.2f show combination of livestock kept per households.

Appendix 7b

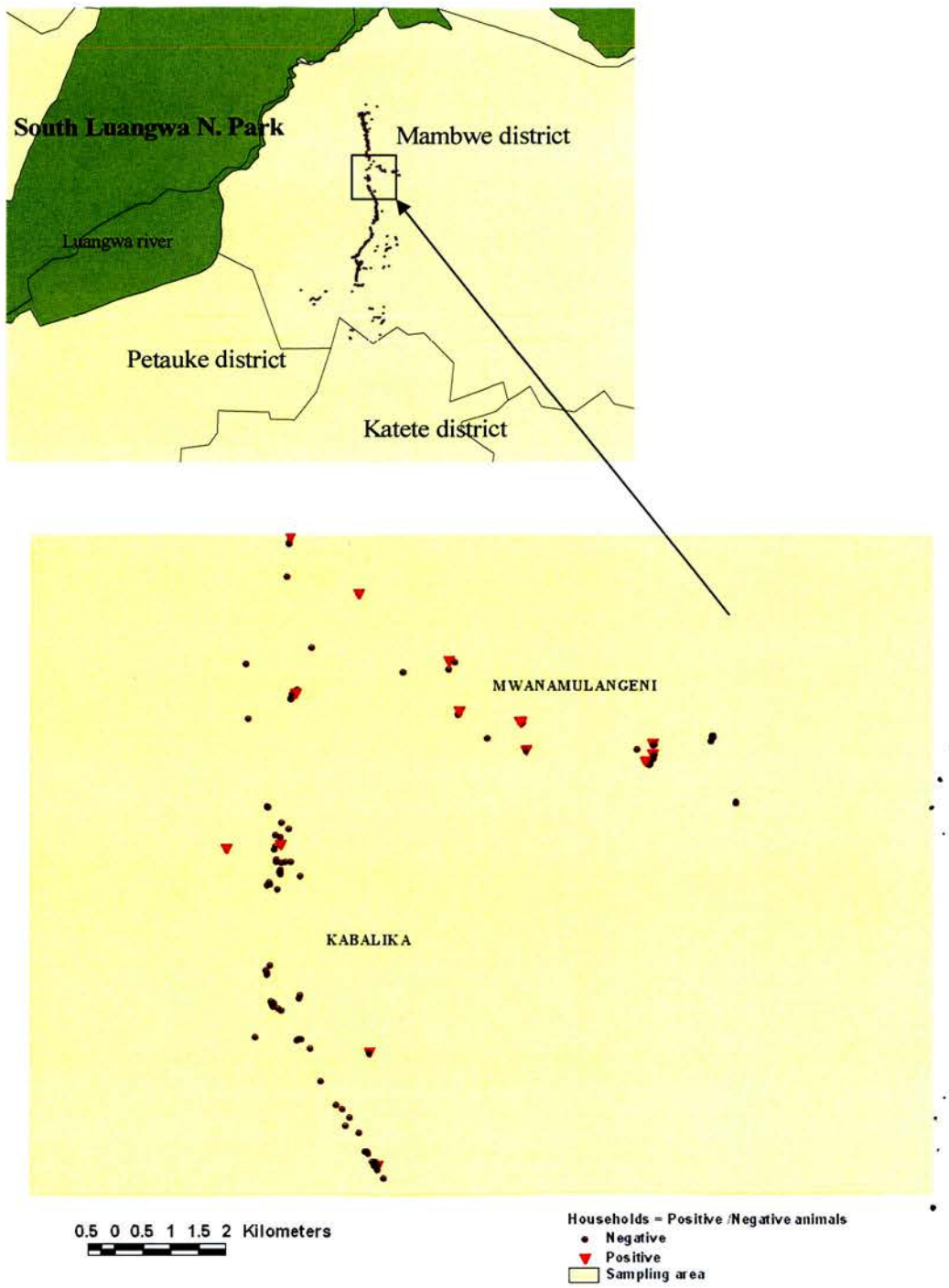
Map 7.1a Zoomed in area 1 shown by a rectangle



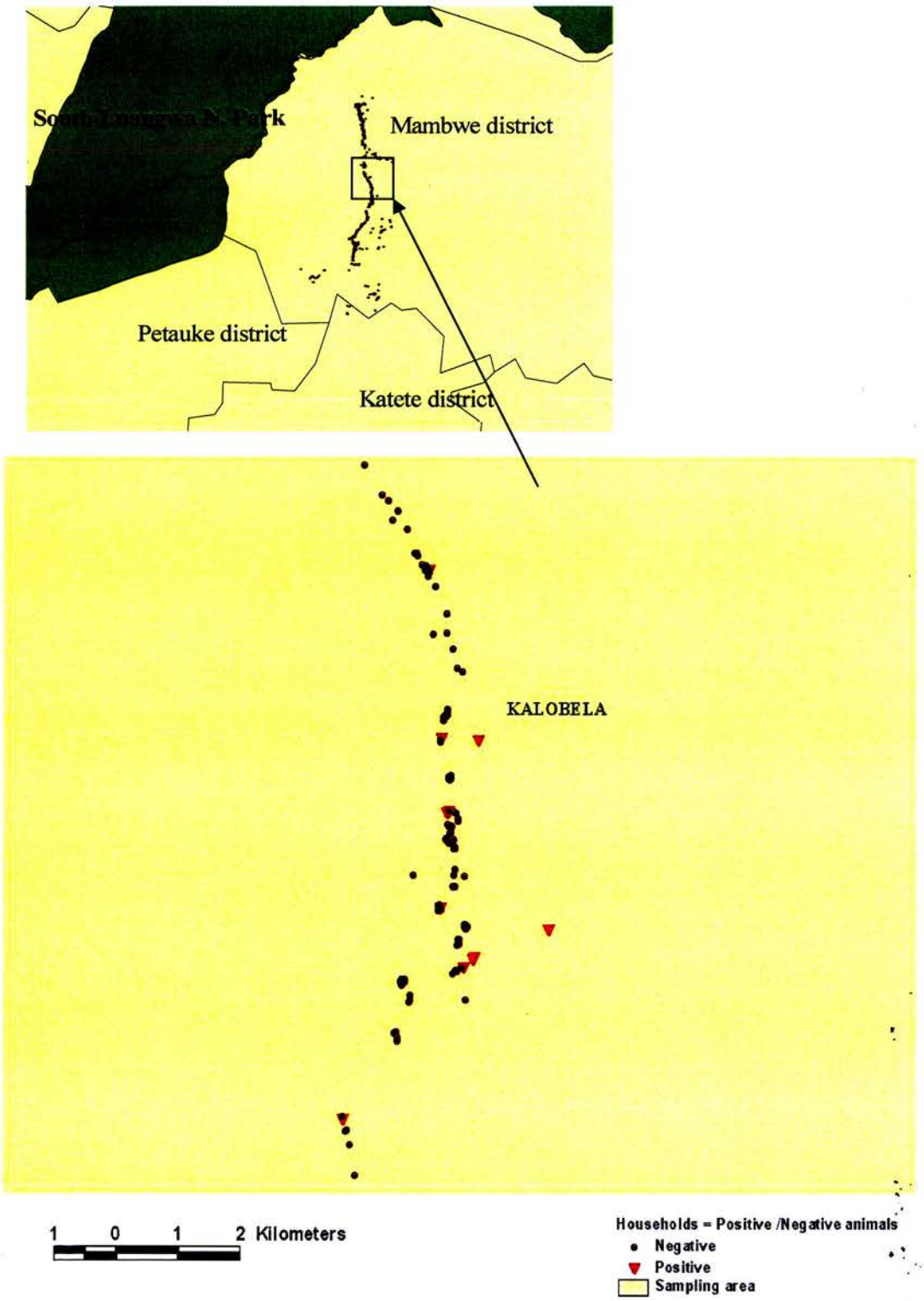
Map 7.1b Zoomed in area 2 shown by a rectangle



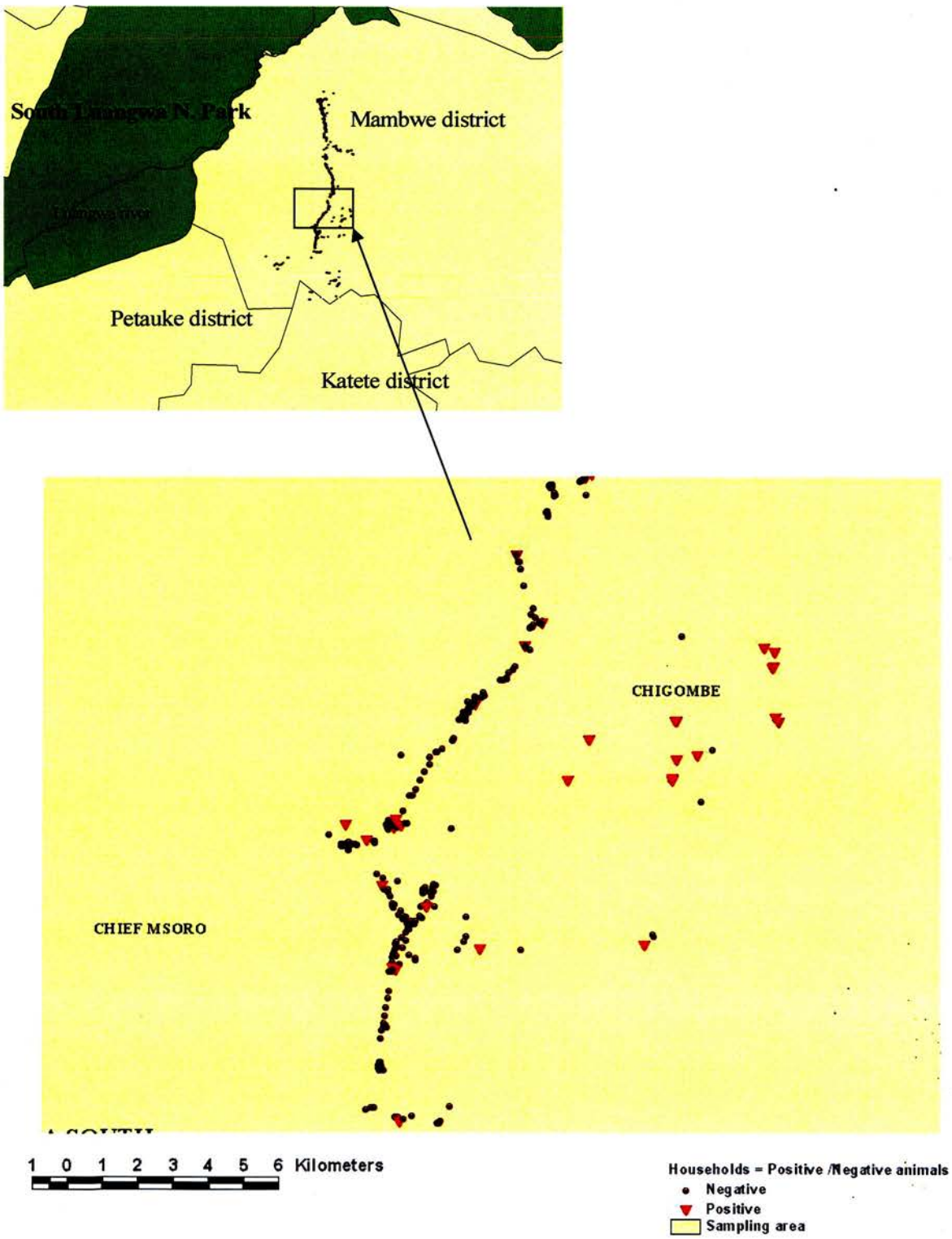
Map 7.1c Zoomed in area 3 shown by a rectangle



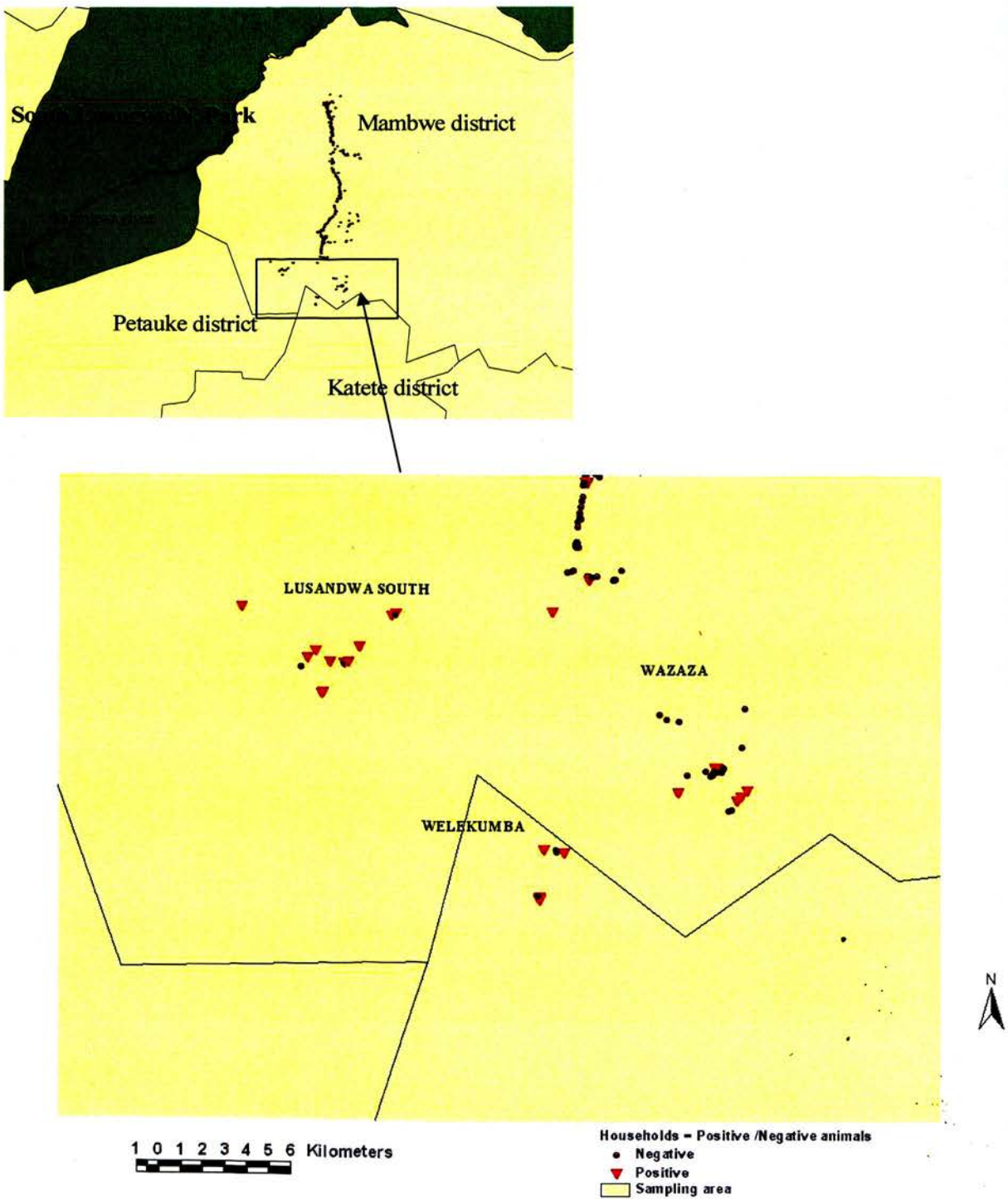
Map 7.1d Zoomed in area 4 shown by a rectangle



Map 7.1e Zoomed in area 5 shown by a rectangle



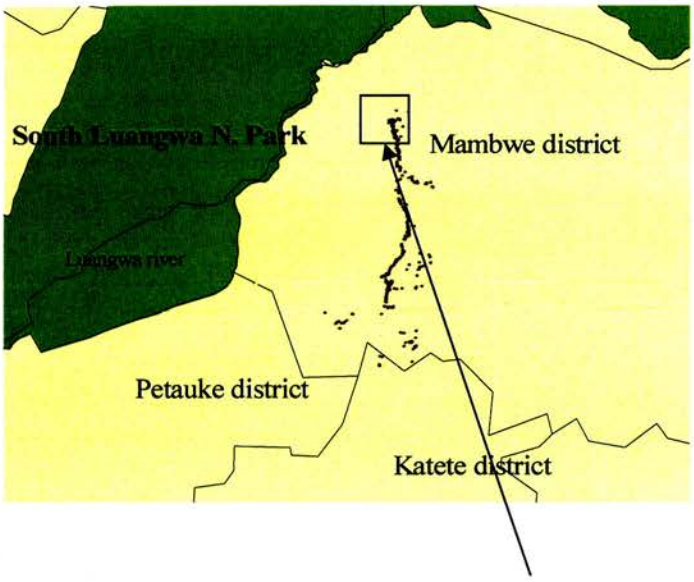
Map 7.1f Zoomed in area 5 shown by a rectangle

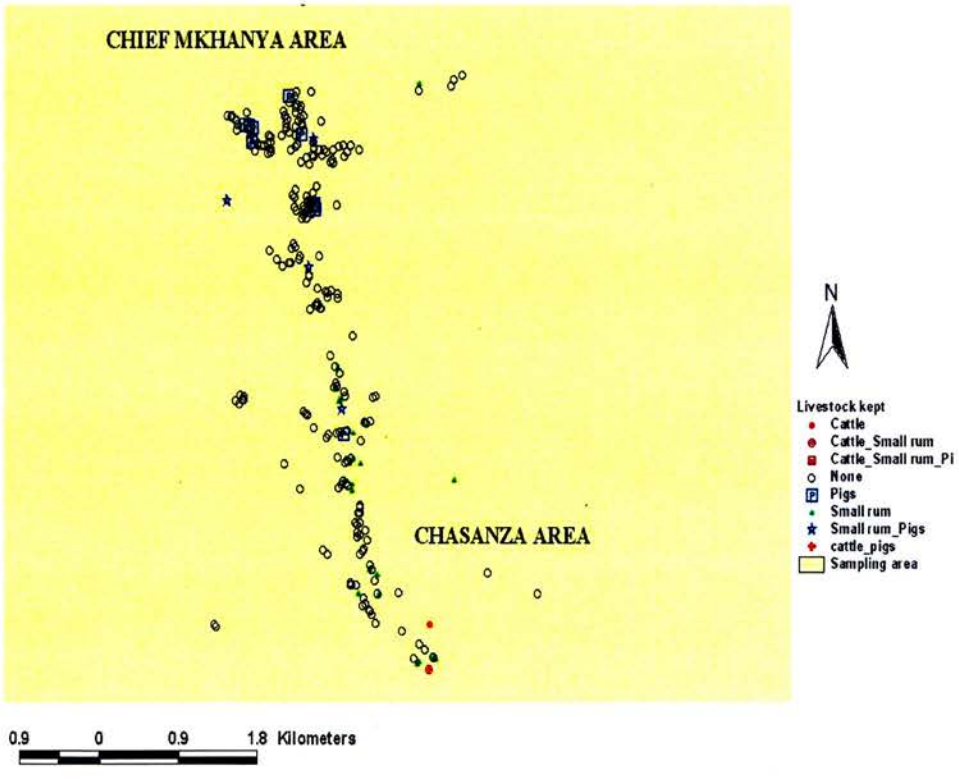


APPENDIX 7

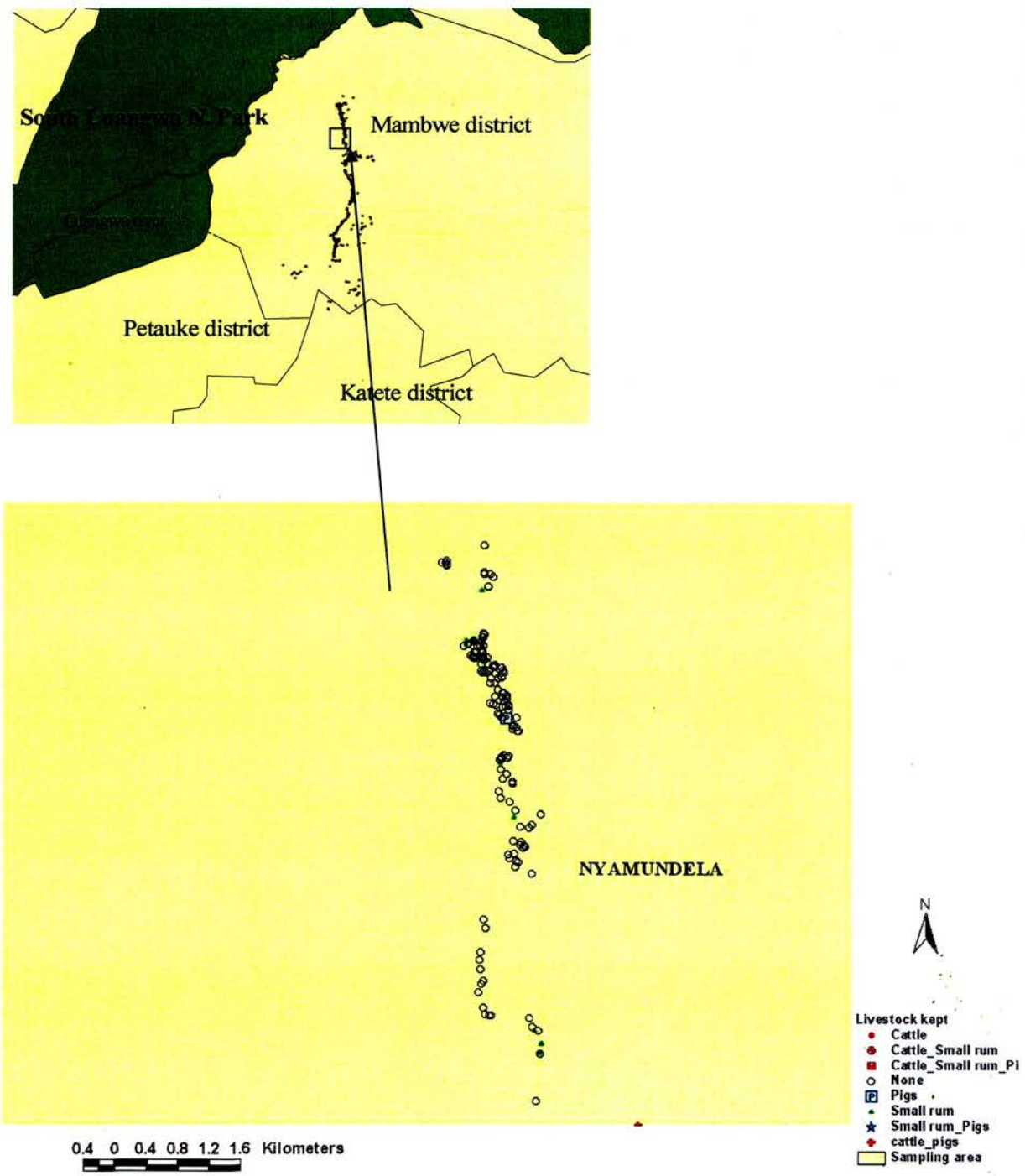
Appendix 7.2

Map 7.2 Zoomed in area 1 shown by a rectangle. Showing combination of livestock kept per household in Mambwe district.

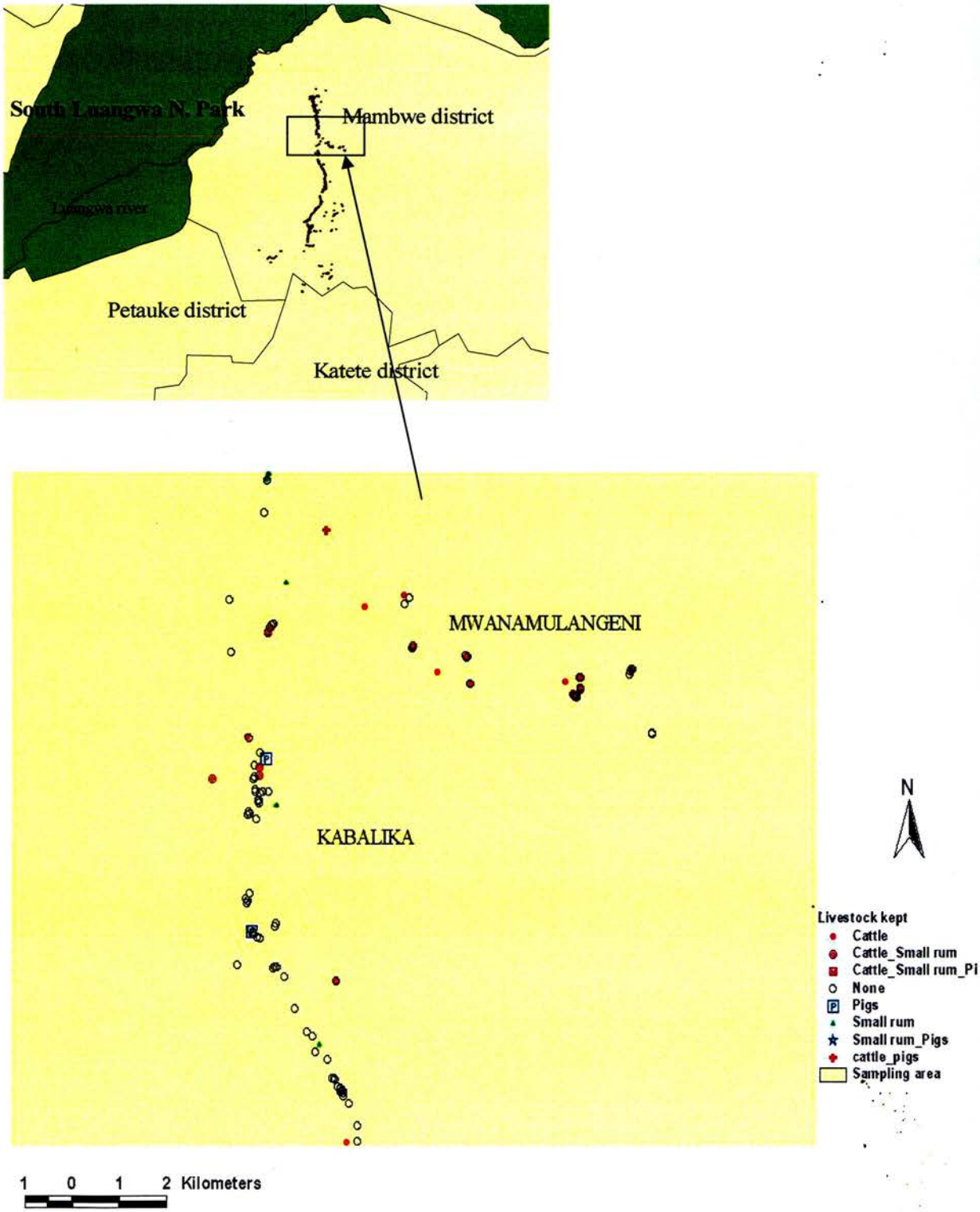




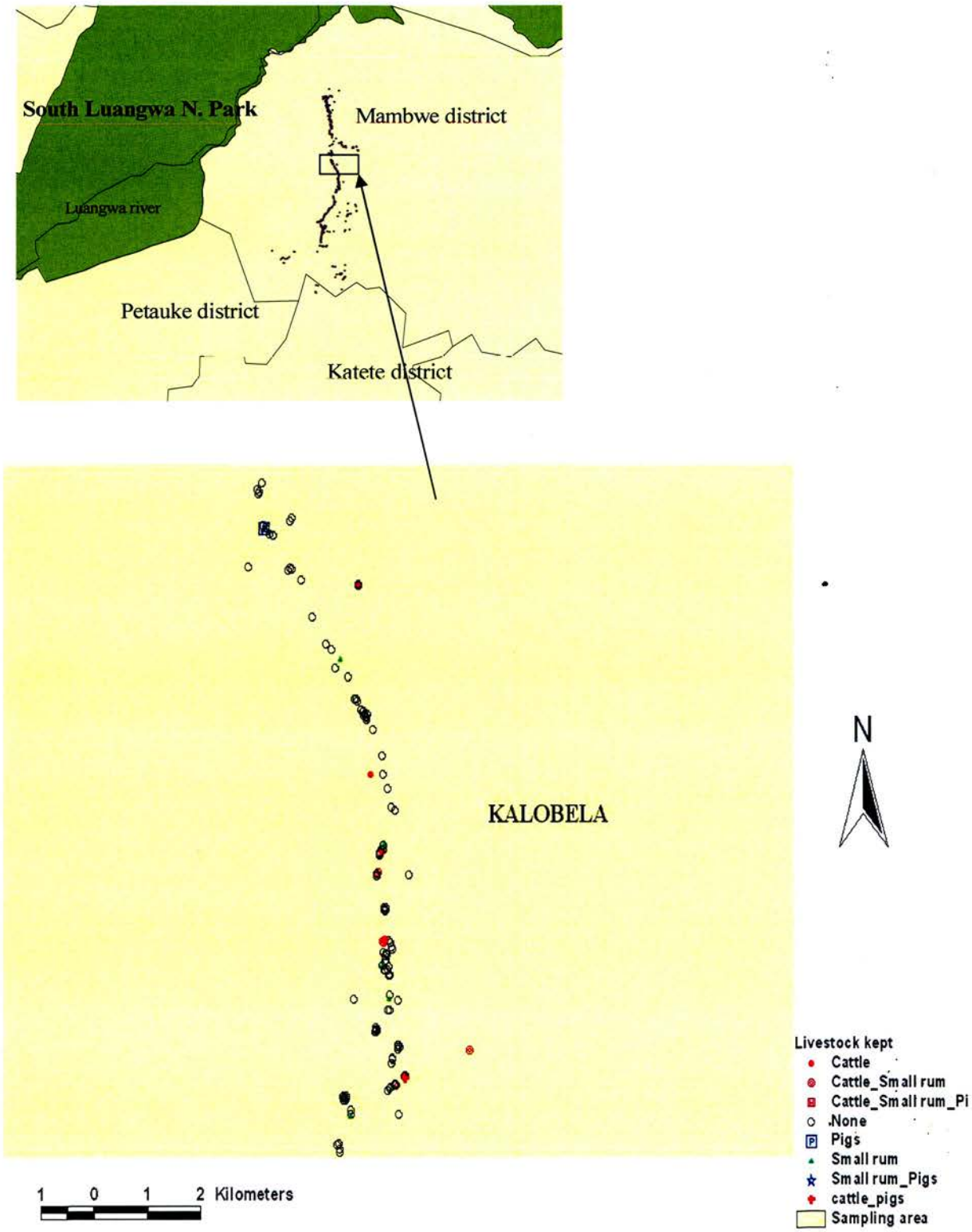
Map 7.2b Zoomed in area 2 shown by a rectangle. Showing combination of livestock kept per household in Mambwe district.



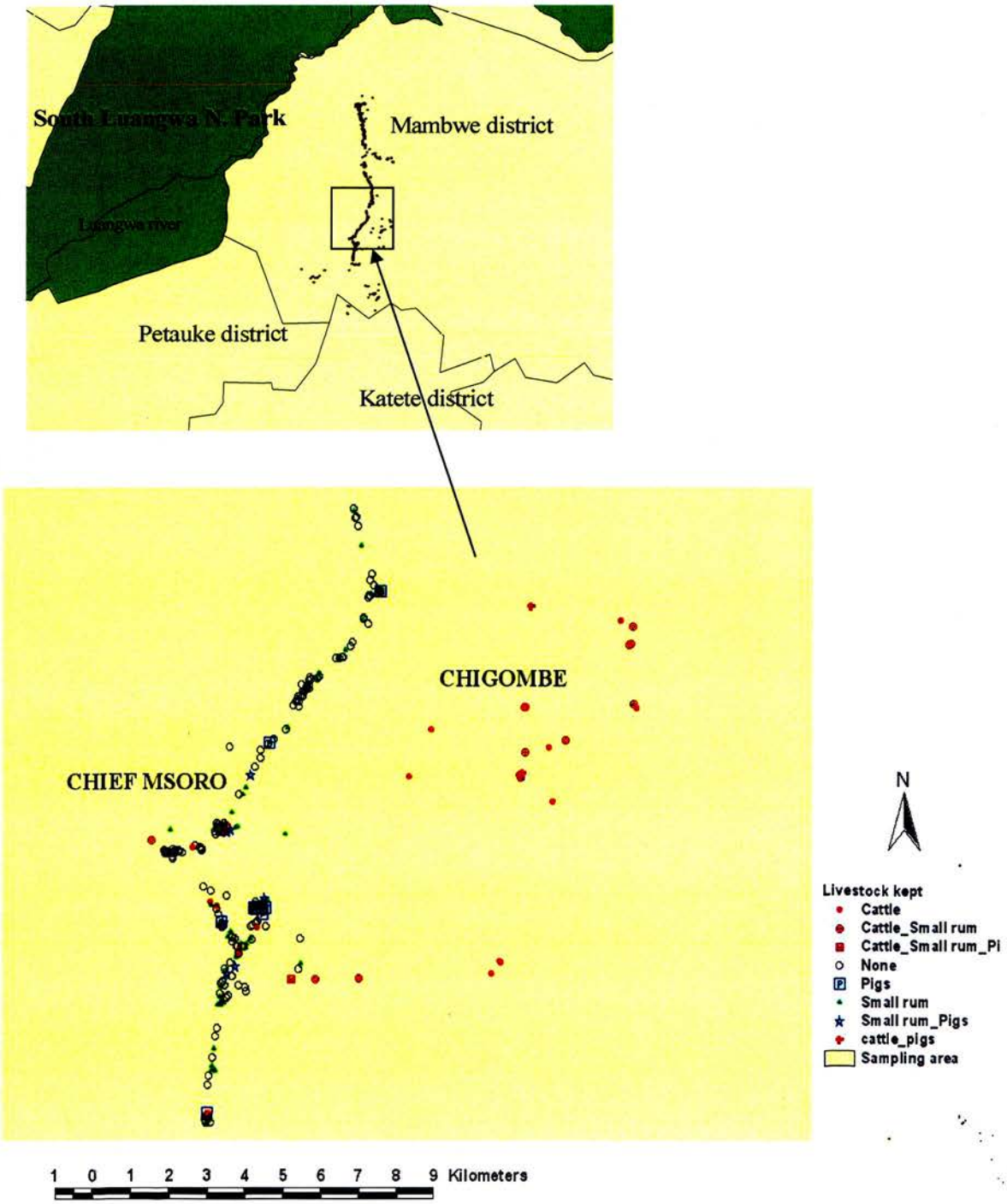
Map 7.2c Zoomed in area 3 shown by a rectangle. Showing combination of livestock kept per household in Mambwe district.



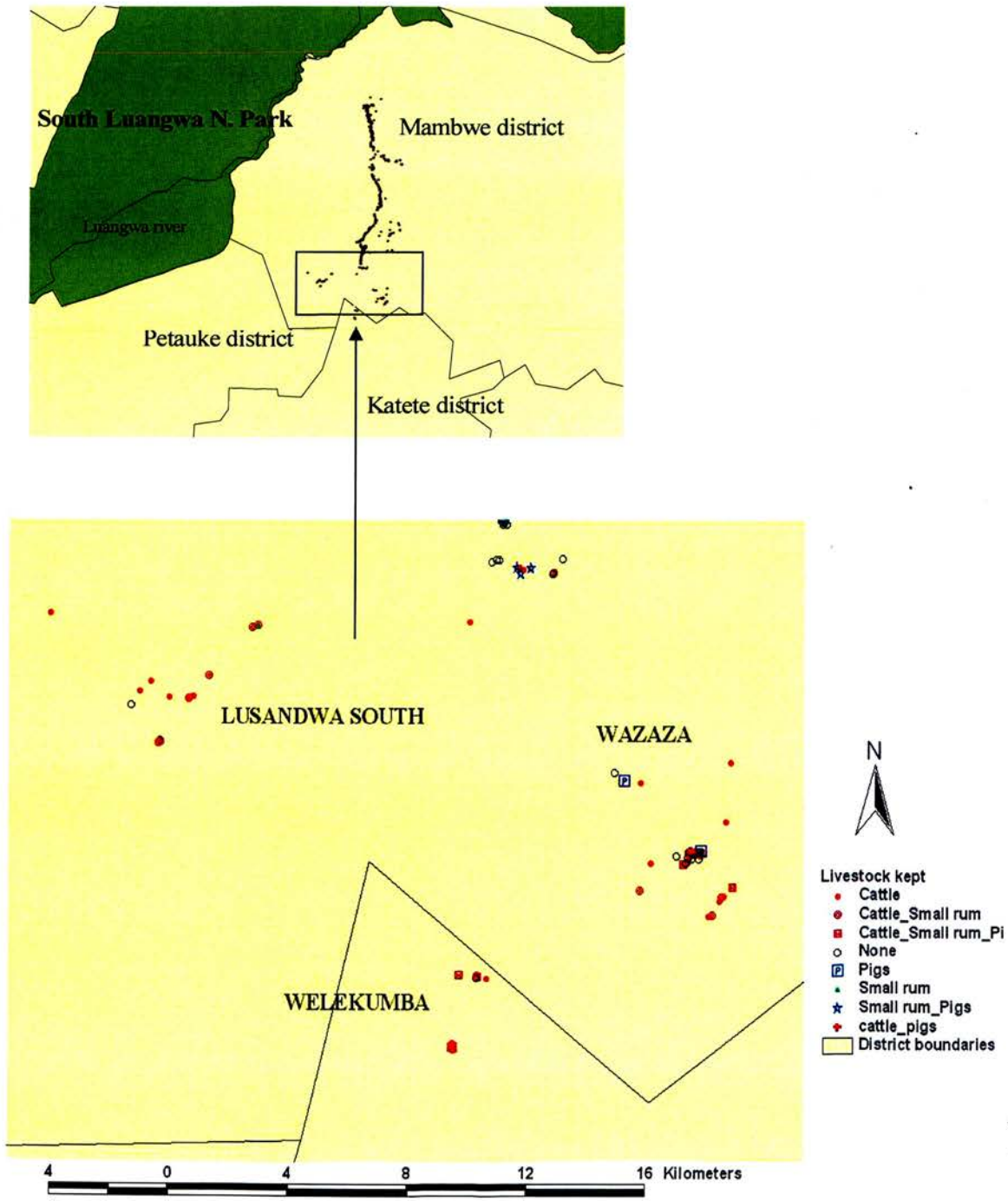
Map 7.2d Zoomed in area 4 shown by a rectangle. Showing combination of livestock kept per household in Mambwe district.



Map 7.2e Zoomed in area 5 shown by a rectangle. Showing combination of livestock kept per household in Mambwe district.



Map 7.2f Zoomed in area 6 shown by a rectangle. Showing combination of livestock kept per household in Mambwe district.



APPENDIX 8

Table 1a: Zero category – *Amblyomma* ticks

	ISMM			Control							
Day	x	N	prop	x	N	prop	χ^2	95%CI		d.f.	p-value
0	187	189	0.9894	167	173	0.9653	1.4404	-0.0124,	0.0606	1	0.2301
28	144	169	0.8521	173	186	0.9301	4.8544	-0.1486,	-0.0075	1	0.0278*
56	77	144	0.5347	97	172	0.5640	0.1654	-0.1457,	0.0873	1	0.6842
84	32	166	0.1928	22	180	0.1222	2.7497	-0.0120,	0.1531	1	0.09727
112	2	151	0.0132	4	154	0.0260	0.1506	-0.0503,	0.0249	1	0.698
140	0	141	0.0000	1	159	0.0063	0	-0.0249,	0.0123	1	1

*** = statistically significant, ISMM = isometamidium chloride**

Table 1b: Low infestation category – *Amblyomma* ticks

	ISMM			Control							
Day	X	N	prop	x	N	prop	χ^2	95%CI		d.f.	p-value
0	2	189	0.0106	6	173	0.0347	1.4404	-0.0606,	0.0124	1	0.2301
28	24	169	0.1420	13	186	0.0699	4.1907	0.0023,	0.1419	1	0.04065*
56	62	144	0.4306	73	172	0.4244	0	-0.1095	0.1218	1	1
84	100	166	0.6024	122	180	0.6778	1.8182	-0.1822,	0.0314	1	0.1775
112	47	151	0.3113	53	154	0.3442	0.24	-0.1447,	0.0789	1	0.6242
140	40	141	0.2837	45	159	0.2830	0	-0.1022,	0.1035	1	1

*** = statistically significant, ISMM = isometamidium chloride**

Table 1c: Medium infestation category - *Amblyomma* ticks

	ISMM			Control							
Day	X	N	Prop	x	N	Prop	χ^2	95%CI		df	p-value
0	0	189	0	0	173	0	0	-0.0220,	0.0200	1	1
28	1	169	0.0059	0	186	0	0.0023	-0.0113,	0.0231	1	0.9617
56	5	144	0.0347	2	172	0.0116	1.011	-0.0172,	0.0634	1	0.3147
84	34	166	0.2048	35	180	0.1944	0.0114	-0.0798,	0.1005	1	0.915
112	64	151	0.4238	60	154	0.3896	0.242	-0.0825,	0.1510	1	0.6228
140	75	141	0.5319	93	159	0.5849	0.6502	-0.1722	0.0662	1	0.4201

ISMM = isometamidium chloride

Table 1d: High infestation category - *Amblyomma* ticks

	ISMM			Control						
Day	X	N	Prop	x	N	Prop	χ^2	95%CI	df	p-value
0	0	189	0	0	173	0	0	-0.0220, 0.0200	1	1
28	0	169	0	0	186	0	0	-0.0200, 0.0200	1	1
56	0	144	0	0	172	0	0	-0.0220, 0.0260	1	1
84	0	166	0	1	180	0.0056	0	-0.0220, 0.0109	1	1
112	38	151	0.2517	37	154	0.2403	0.0096	-0.0918, 0.1146	1	0.9219
140	26	141	0.1844	20	159	0.1258	1.5517	-0.0303, 0.1475	1	0.2129

Table 2a. Zero category – *Amblyomma* ticks

	Pour-On			Control						
Day	X	N	prop	x	N	prop	χ^2	95%CI	df	p-value
0	160	163	0.9816	167	173	0.9653	0.3428	-0.0239, 0.0564	1	0.558
28	162	166	0.9759	173	186	0.9301	3.0681	-0.0033, 0.0949	1	0.080
56	119	166	0.7169	97	172	0.5640	7.9131	0.0461, 0.2598	1	0.005*
84	55	166	0.3313	22	180	0.1222	20.6322	0.1172, 0.3010	1	0.001*
112	7	153	0.0458	4	154	0.0260	0.3908	-0.0283 0.0678	1	0.532
140	14	158	0.0886	1	159	0.0063	10.1565	0.0300, 0.1346	1	0.001*

*** = Statistically significant**

Table 2b. Low infestation category – *Amblyomma* ticks

	PourOn			Control						
Day	X	N	prop	x	N	prop	χ^2	95%CI	df	p-value
0	3	163	0.0184	6	173	0.0347	0.3428	-0.0564, 0.0239	1	0.5582
28	4	166	0.0241	13	186	0.0699	3.0681	-0.0949, 0.0033	1	0.07984
56	147	166	0.8855	73	172	0.4244	77.0303	0.3669, 0.5554	1	0.0001*
84	108	166	0.6506	122	180	0.6778	0.1772	-0.1326, 0.0782	1	0.6738
112	98	153	0.6405	53	154	0.3442	25.799	0.1830 0.4097	1	0.0001*
140	96	158	0.6076	45	159	0.2830	32.5061	0.2148, 0.4343	1	0.0001*

*** = Statistically significant**

Table 2c. Medium infestation category – *Amblyomma* ticks

	PourOn			Control						
Day	**X**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**	**df**	**p-value**
0	0	163	0	0	173	0	0	-0.022, 0.023	1	1
28	0	166	0	0	186	0	0	-0.020, 0.023	1	1
56	0	166	0	2	172	0.0116	0.468	-0.0336, 0.0103	1	0.4939
84	3	166	0.0181	35	180	0.1944	25.7034	-0.2434, -0.1093	1	0.0001*
112	46	153	0.3006	60	154	0.3896	2.3075	-0.2013, 0.0234	1	0.1287
140	47	158	0.2975	93	159	0.5849	25.3992	-0.3984, -0.1765	1	0.0001*

*** = Statistically significant**

Table 2d High infestation category – *Amblyomma* ticks

	Pour-on			Control						
Day	**X**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**	**df**	**p-value**
0	0	163	0	0	173	0	0	-0.022, 0.023	1	1
28	0	166	0	0	186	0	0	-0.020, 0.023	1	1
56	0	166	0	0	172	0	0	-0.032, 0.017	1	1
84	0	166	0	1	180	0.0056	0	-0.0220, 0.0109	1	1
112	2	153	0.0131	37	154	0.2403	33.7016	-0.3035, -0.1508	1	0.0001*
140	1	158	0.0063	20	159	0.1258	16.4019	-0.1788 -0.0601	1	0.0001*

*** = Statistically significant**

Table 3a. Zero category – *Amblyomma* ticks

	RA			Control						
Day	x	N	prop	x	N	prop	χ^2	95%CI		df	p-value
0	200	203	0.9852	167	173	0.9653	0.8464	-0.0174,	0.0572	1	0.3576
28	161	182	0.8846	173	186	0.9301	1.7602	-0.110 1,	0.0191	1	0.1846
56	135	194	0.6959	97	172	0.5640	6.2803	0.0280,	0.2358	1	0.01221*
84	73	174	0.4195	22	180	0.1222	38.3332	0.2041,	0.3905	1	0.0001*
112	29	180	0.1611	4	154	0.0260	15.538	0.0698,	0.2004	1	0.0001*
140	38	158	0.2405	1	159	0.0063	38.1525	0.1601,	0.3083	1	0.0001*

*** = Statistically significant RA = restricted application of insecticide**

Table 3b. Low infestation category – *Amblyomma* ticks

	RA			Control						
Day	x	N	prop	x	N	prop	χ^2	95%CI		d.f.	p-value
0	3	203	0.0148	6	173	0.0347	0.8464	-0.0572,	0.0174	1	0.3576
28	21	182	0.1154	13	186	0.0699	1.7602	-0.0191,	0.1101	1	0.1846
56	59	194	0.3041	73	172	0.4244	5.2118	-0.2240,	-0.0166	1	0.02243*
84	94	174	0.5402	122	180	0.6778	6.4708	-0.2439,	-0.0312	1	0.01097*
112	111	180	0.6167	53	154	0.3442	23.5829	0.1632,	0.3818	1	0.0001*
140	92	158	0.5823	45	159	0.2830	27.7146	0.1889,	0.4096	1	0.0001*

*** = Statistically significant RA = restricted application of insecticide**

Table 3c. Medium infestation category – *Amblyomma* ticks

	Spray			Control						
Days	x	N	Prop	x	N	Prop	χ^2	95%CI		d.f.	p-value
0	0	203	0	0	173	0	0	-0.022,	0.019	1	1
28	0	182	0	0	186	0	0	-0.020,	0.021	1	1
56	0	194	0.0000	2	172	0.0116	0.6332	-0.0331,	0.0099	1	0.4262
84	7	174	0.0402	35	180	0.1944	18.6743	-0.2246,	-0.0838	1	0.0001*
112	40	180	0.2222	60	154	0.3896	10.3023	-0.2715,	-0.0633	1	0.0013*
140	28	158	0.1772	93	159	0.5849	54.0986	-0.5110,	-0.3044	1	0.0001*

Table 3d. High infestation category – *Amblyomma* ticks

	Spray			Control							
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	0	203	0	0	173	0	0	-0.022,	0.019	1	1
28	0	182	0	0	186	0	0	-0.020,	0.021	1	1
56	0	194	0	0	172	0	0	-0.022,	0.019	1	1
84	0	174	0	1	180	0.0056	0	-0.0220,	0.0109	1	1
112	0	180	0	37	154	0.2403	46.226	-0.3138,	-0.1668	1	0.0001*
140	0	158	0	20	159	0.1258	19.1379	-0.1836,	-0.0679	1	0.0001*

*** = Statistically significant RA = restricted application of insecticide**

APPENDIX 9

Table 1a Zero category – *Boophilus* ticks

	ISSM			Control							
Days	**x**		**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f**	**p-value**
0	5	189	0.0265	8	188	0.0426	0.3298	-0.0582,	0.0260	1	0.5658
28	21	169	0.1243	22	186	0.1183	0.0001	-0.0677	0.0796	1	0.9923
56	33	144	0.2292	25	172	0.1453	3.1365	-0.0091,	0.1767	1	0.0766
84	58	166	0.3494	32	180	0.1778	12.339	0.0743,	0.2690,	1	0.0004*
112	60	151	0.3974	70	154	0.4545	0.7994	-0.1746,	0.0602	1	0.3713
140	71	141	0.5035	66	159	0.4151	2.0134	-0.0308,	0.2077	1	0.1559

***= Statistically Significant ISMM = isometamidium chloride**

Table 1b. Low infestation category – *Boophilus* ticks

	ISMM			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	80	189	0.4233	95	188	0.5053	2.2312	-0.1877,	0.0236	1	0.1352
28	111	169	0.1243	137	186	0.1183	2.3095	-0.1810,	0.0214	1	0.1286
56	78	144	0.5417	97	172	0.5640	0.0803	-0.1387,	0.0942	1	0.7770
84	93	166	0.5602	128	180	0.7111	7.8772	-0.2571,	-0.0447	1	0.0050*
112	73	151	0.4834	69	154	0.4481	0.2548	-0.0831,	0.1538	1	0.6137
140	55	141	0.3901	49	159	0.3082	1.8661	-0.0326,	0.1964	1	0.1719

***= Statistically Significant ISMM = isometamidium chloride**

Table 1c. Medium infestation category – *Boophilus* ticks

	ISMM			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	62	189	0.3280	64	188	0.3404	0.0212	-0.1129,	0.0881	1	0.8842
28	30	169	0.1775	27	186	0.1452	0.4686	-0.0499,	0.1146	1	0.4936
56	24	144	0.1667	33	172	0.1919	0.4686	-0.0499,	0.1146	1	0.4936
84	14	166	0.0843	19	180	0.1056	0.2383	-0.0886,	0.0462	1	0.6255
112	15	151	0.0993	15	154	0.0974	0	-0.0668,	0.0707	1	1.0000
140	14	141	0.0993	37	159	0.2327	8.5051	-0.2222,	-0.0445	1	0.0035*

***= Statistically Significant ISMM = isometamidium chloride**

Table 1d. High infestation category – *Boophilus* ticks

	ISMM			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	42	189	0.2222	21	188	0.1117	7.4963	0.0307,	0.1902	1	0.0062*
28	7	169	0.0414	0	186	0.0000	5.8623	0.0057,	0.0771	1	0.0155*
56	9	144	0.0625	17	172	0.0988	0.9316	-0.1023,	0.0296	1	0.3344
84	1	166	0.0060	1	180	0.0056	0	-0.0160,	0.0169	1	1.0000
112	3	151	0.0199	0	154	0.0000	1.3867	-0.0089,	0.0486	1	0.2390
140	1	141	0.0071	7	159	0.0440	2.6332	-0.0783,	0.0045	1	0.1046

***= Statistically Significant ISMM = isometamidium chloride**

Table 2a. Zero category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	7	163	0.0429	8	188	0.0426	0	-0.0424,	0.0432	1	1
28	68	159	0.4277	22	186	0.1183	40.9673	0.2137,	0.4050	1	<0.001*
56	68	166	0.4096	25	172	0.1453	28.2742	0.1668,	0.3617	1	<0.001*
84	104	166	0.6265	32	180	0.1778	71.0207	0.3505,	0.5469	1	<0.001*
112	104	153	0.6797	70	154	0.4545	14.9473	0.1107,	0.3396	1	<0.001*
140	116	158	0.7342	66	159	0.4151	31.708	0.2097,	0.4284	1	<0.001*

***=Statistically Significant**

Table 2b. Low infestation category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	86	163	0.5276	95	188	0.5053	0.0959	-0.0882,	0.1328	1	0.7568
28	89	159	0.4277	137	186	0.1183	11.0906	-0.2824,	-0.0711	1	0.0008*
56	92	166	0.5542	97	172	0.5639	0.005	-0.1215,	0.1020	1	0.9437
84	62	166	0.3735	128	180	0.7111	38.4063	-0.4423,	-0.2328	1	<0.001*
112	49	153	0.3203	69	154	0.4480	4.7704	-0.2421,	-0.0134	1	0.0289*
140	40	158	0.2532	49	159	0.3081	0.9309	-0.1600,	0.0500	1	0.3346

Table 2c. Medium infestation category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-val**
0	56	163	0.3435	64	188	0.3404	0	-0.0995,	0.1057	1	1
28	2	159	0.0125	27	186	0.1451	17.8871	-0.1919,	-0.0732	1	<0.00
56	6	166	0.0361	33	172	0.1918	18.5705	-0.2269,	-0.0844	1	<0.00
84	0	166	0	19	180	0.1055	16.5622	-0.1562,	-0.0548	1	<0.00
112	0	153	0	15	154	0.0974	13.6423	-0.1507,	-0.0440	1	<0.00
140	2	158	0.012658	37	159	0.2327	33.5555	-0.2943,	0.1457	1	<0.00

***=Statistically Significant**

Table 2d. High infestation category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	***p*-value**
0	14	163	0.0858	21	188	0.1117	0.3923	-0.0938,	0.0421	1	0.5311
28	0	159	0	0	186	0	0	-0. 0200,	0.024	1	1
56	0	166	0	17	172	0.0988	15.2687	-0.1493,	-0.0483	1	<0.001
84	0	166	0	1	180	0.0055	0	-0.0219,	0.0108	1	1
112	0	153	0	0	154	0	0	-0.024,	0.024	1	1
140	0	158	0	7	159	0.0440	5.2204	-0.0822,	-0.0058	1	0.0223

***=Statistically Significant**

Table 2a. Zero category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	7	163	0.0429	8	188	0.0426	0	-0.0424,	0.0432	1	1
28	68	159	0.4277	22	186	0.1183	40.9673	0.2137,	0.4050	1	<0.001*
56	68	166	0.4096	25	172	0.1453	28.2742	0.1668,	0.3617	1	<0.001*
84	104	166	0.6265	32	180	0.1778	71.0207	0.3505,	0.5469	1	<0.001*
112	104	153	0.6797	70	154	0.4545	14.9473	0.1107,	0.3396	1	<0.001*
140	116	158	0.7342	66	159	0.4151	31.708	0.2097,	0.4284	1	<0.001*

*=Statistically Significant

Table 2b. Low infestation category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	86	163	0.5276	95	188	0.5053	0.0959	-0.0882,	0.1328	1	0.7568
28	89	159	0.4277	137	186	0.1183	11.0906	-0.2824,	-0.0711	1	0.0008*
56	92	166	0.5542	97	172	0.5639	0.005	-0.1215,	0.1020	1	0.9437
84	62	166	0.3735	128	180	0.7111	38.4063	-0.4423,	-0.2328	1	<0.001*
112	49	153	0.3203	69	154	0.4480	4.7704	-0.2421,	-0.0134	1	0.0289*
140	40	158	0.2532	49	159	0.3081	0.9309	-0.1600,	0.0500	1	0.3346

Table 2c. Medium infestation category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-valu**
0	56	163	0.3435	64	188	0.3404	0	-0.0995,	0.1057	1	1
28	2	159	0.0125	27	186	0.1451	17.8871	-0.1919,	-0.0732	1	<0.001
56	6	166	0.0361	33	172	0.1918	18.5705	-0.2269,	-0.0844	1	<0.001
84	0	166	0	19	180	0.1055	16.5622	-0.1562,	-0.0548	1	<0.001
112	0	153	0	15	154	0.0974	13.6423	-0.1507,	-0.0440	1	<0.001
140	2	158	0.012658	37	159	0.2327	33.5555	-0.2943,	0.1457	1	<0.001

*=Statistically Significant

Table 2d. High infestation category – *Boophilus* ticks

	Pour-on			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	***p*-valu**
0	14	163	0.0858	21	188	0.1117	0.3923	-0.0938,	0.0421	1	0.5311
28	0	159	0	0	186	0	0	-0. 0200,	0.024	1	1
56	0	166	0	17	172	0.0988	15.2687	-0.1493,	-0.0483	1	<0.001
84	0	166	0	1	180	0.0055	0	-0.0219,	0.0108	1	1
112	0	153	0	0	154	0	0	-0.024,	0.024	1	1
140	0	158	0	7	159	0.0440	5.2204	-0.0822,	-0.0058	1	0.0223

***=Statistically Significant**

Table 3a. Zero category – *Boophilus* ticks

	RA			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	44	203	0.2167	8	188	0.0426	24.1978	0.1054,	0.2429	1	<0.001*
28	71	182	0.3901	22	186	0.1183	34.5675	0.1816,	0.3619	1	<0.001*
56	83	194	0.4278	25	172	0.1453	33.6305	0.1897,	0.3752	1	<0.001*
84	130	174	0.7471	32	180	0.1778	113.2663	0.4783,	0.6603	1	<0.001*
112	153	180	0.8500	70	154	0.4545	56.7245	0.2950,	0.4958	1	<0.001*
140	120	158	0.7595	66	159	0.4151	37.3589	0.2365,	0.4522	1	<0.001*

***=Statistically significant RA = Restricted application of insecticide**

Table 3b. Low infestation category – *Boophilus* ticks

	RA			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	105	203	0.5172	95	188	0.5053	0.0181	-0.0923,	0.1162	1	0.893
28	107	182	0.3901	137	186	0.1183	8.4446	-0.2495,	-0.0477	1	0.004*
56	105	194	0.5412	97	172	0.5640	0.1095	-0.1302,	0.0847	1	0.741
84	44	174	0.2529	128	180	0.7111	6.1607	-0.1793,	-0.0206	1	0.013*
112	26	180	0.1444	69	154	0.4481	36.1106	-0.4034,	-0.2037	1	<0.001*
140	37	158	0.2342	49	159	0.3082	1.8367	-0.1778,	0.0298	1	0.175

***=Statistically significant RA = Restricted application of insecticide**

Table 3c. Medium infestation category – *Boophilus* ticks

	RA			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	49	203	0.2414	64	188	0.3404	4.1904	-0.1939,	-0.0041	1	0.041*
28	4	182	0.0220	27	186	0.1452	16.5328	-0.1835,	-0.0628	1	<0.001*
56	6	194	0.0309	33	172	0.1919	23.1404	-0.2301,	-0.0917	1	<0.001*
84	0	174	0.0000	19	180	0.1056	17.3858	-0.1560,	-0.0550	1	<0.001*
112	1	180	0.0056	15	154	0.0974	13.4028	-0.1459,	-0.0377	1	0.0002*
140	1	158	0.0063	37	159	0.2327	36.3774	-0.2995,	-0.1532	1	<0.001*

***=Statistically significant RA = Restricted application of insecticide**

Table 3d. High infestation category – *Boophilus* ticks

	RA			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**df**	**p-value**
0	5	203	0.0246	21	188	0.1117	10.5597	-0.1420	-0.0321	1	0.001*
28	0	182	0	0	186	0	0	-0.020,	0.021	1	1
56	0	194	0	17	172	0.0988	17.9388	-0.1489	-0.0488	1	<0.001*
84	0	174	0	1	180	0.0056	0	-0.0220	0.0108	1	1
112	0	180	0	0	154	0	0	-0.024,	0.0210	1	1
140	0	158	0	7	159	0.0440	5.2204	-0.0822	-0.0058	1	0.022*

***=Statistically significant RA = Restricted application of insecticide**

APPENDIX 10

Table 1a. Zero category – *Rhipicephalus* ticks

	ISMM			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	149	189	0.7884	110	173	0.6358	9.5859	0.0546,	0.2504	1	0.001*
28	113	169	0.6686	64	186	0.3441	36.0214	0.2204,	0.4286	1	<0.001*
56	16	144	0.1111	12	172	0.0698	1.1865	-0.0289,	0.1116	1	0.276
84	10	166	0.0602	5	180	0.0278	1.4815	-0.0167,	0.0816	1	0.224
112	96	151	0.6358	78	154	0.5065	4.6853	0.0125,	0.2459	1	0.030*
140	95	141	0.6738	63	159	0.3962	21.9899	0.1623,	0.3927	1	<0.001*

*= Statistically significant ISMM = isometamidium chloride

Table 1b. Low infestation category – *Rhipicephalus* ticks

	ISMM			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	38	189	0.2011	57	173	0.3295	7.0467	-0.2243,	-0.0324	1	0.008*
28	54	169	0.3195	107	186	0.5753	21.9261	-0.3596,	-0.1480	1	<0.001*
56	103	144	0.7153	83	172	0.4826	12.3865	0.0886,	0.3086	1	0.0004*
84	114	166	0.6867	93	180	0.5167	9.6983	0.0627,	0.2773	1	0.002*
112	55	151	0.3642	75	154	0.4870	4.2107	-0.2394,	-0.0069	1	0.040*
140	46	141	0.3262	95	159	0.5975	20.9964	-0.3865,	-0.1559	1	<0.001*

*= Statistically significant ISMM = isometamidium chloride

Table 1c. Medium infestation category – *Rhipicephalus* ticks

	ISMM			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	2	189	0.0106	6	173	0.0347	1.4404	-0.0605,	0.01235	1	0.2301
28	2	169	0.0118	15	186	0.0806	7.7482	-0.1168,	-0.0207	1	0.005*
56	23	144	0.1597	70	172	0.4070	21.8965	-0.3483,	-0.1461	1	<0.001*
84	42	166	0.2530	69	180	0.3833	6.1464	-0.2331,	-0.0274	1	0.013*
112	0	151	0	1	154	0.0065	0	-0.0256,	0.0126	1	1
140	0	141	0	1	159	0.0063	0	-0.0248,	0.0122	1	1

*= Statistically significant ISMM = isometamidium chloride

Table 2d. High infestation category – *Rhipicephalus* ticks

	ISMM			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	0	189	0	0	173	0	0	-0.020,	0.0200	1	1
28	0	169	0	0	186	0	0	-0.020,	0.0220	1	1
56	2	144	0.0139	7	172	0.0407	1.1823	-0.0683	0.0147	1	0.2769
84	0	166	0	13	180	0.0722	0	-0.021,	0.023	1	1
112	0	151	0	0	154	0	0	-0.024,	0.025	1	1
140	0	141	0	0	159	0	0	-0.024,	0.027	1	1

***= Statistically significant ISMM = isometamidium chloride**

Table 2a. Zero infestation category – *Rhipicephalus* ticks

	Pour-on			Control							
Days	x	N	prop	x	N	prop	χ^2	95%CI		df	p-value
0	120	163	0.7362	110	173	0.6358	3.4633	-0.0041,	0.2048	1	0.063
28	148	159	0.9308	64	186	0.3441	122.1076	0.5020,	0.6714	1	< 0.001*
56	64	166	0.3855	12	172	0.0698	46.5326	0.2266,	0.4049	1	< 0.001*
84	43	166	0.2590	5	180	0.0278	36.7427	0.1546,	0.3079	1	< 0.001*
112	110	153	0.7190	78	154	0.5065	13.7136	0.0996,	0.3253	1	0.0002*
140	110	158	0.6962	63	159	0.3962	27.6449	-0.4106,	-0.1893	1	< 0.001*

***= Statistically significant**

Table 2b. Low infestation category – *Rhipicephalus* ticks

	Pour-on			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**df**	**p-value**
0	39	163	0.2393	57	173	0.3295	2.9196	-0.1920,	0.0116	1	0.087
28	10	159	0.9308	107	186	0.5753	98.1388	-0.5986,	-0.4261	1	<0.001*
56	96	166	0.5783	83	172	0.4826	2.7366	-0.0160,	0.2075	1	0.098
84	115	166	0.6928	93	180	0.5167	10.4476	0.0690,	0.28316	1	0.001*
112	43	153	0.2810	75	154	0.4870	12.9029	-0.3188,	-0.0938	1	0.0003*
140	47	158	0.2975	95	159	0.5975	27.6449	-0.4106,	-0.1893	1	<0.001*

***= Statistically significant**

Table 2c. Medium infestation category – *Rhipicephalus* ticks

	Pour-on			Control							
Days	**x**	**N**	**Prop**	**x**		**Prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	4	163	0.0245	6	173	0.0347	0.0509	-0.0522,	0.0319	1	0.822
28	1	159	0.0063	15	186	0.0806	9.1009	-0.1212,	-0.0275	1	0.003*
56	6	166	0.0361	70	172	0.4070	64.5383	-0.4557,	-0.2861	1	<0.001*
84	8	166	0.0482	69	180	0.3833	54.1416	-0.4190,	-0.2512	1	<0.001*
112	0	153	0	1	154	0.0065	0	-0.0256,	0.0126	1	1
140	1	158	0.0063	1	159	0.0063	0	-0.0174,	0.0175	1	1

*= Statistically significant

Table 2d. High infestation category – *Rhipicephalus* ticks

	Pour-on			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**χ^2**	**95%CI**		**df**	**p-value**
0	0	163	0	0	173	0	0	-0.022,	0.023	1	1
28	0	159	0	0	186	0	0	-0.020,	0.024	1	1
56	0	166	0	7	172	0.0406	5.0379	-0.0761,	-0.0052	1	0.025*
84	0	166	0	13	180	0.0722	10.5397	-0.1158,	-0.0286		0.001*
112	0	153	0	0	154	0	0	-0.024,	0.024	1	1
140	0	158	0	0	159	0	0	-0.024,	0.024	1	1

*= Statistically significant

Table 3a. Zero category – *Rhipicephalus* ticks

	RA			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	**χ^2**	**95%CI**		**d.f.**	**p-value**
0	175	203	0.8621	110	173	0.6358	24.8397	0.1349,	0.3175	1	<0.001*
28	133	182	0.7308	64	186	0.3441	53.7507	0.2873,	0.4860	1	<0.001*
56	86	194	0.4433	12	172	0.0698	62.988	0.2884,	0.4586	1	<0.001*
84	54	174	0.3103	5	180	0.0278	48.8479	0.2041,	0.3610	1	<0.001*
112	129	180	0.7167	78	154	0.5065	14.6777	0.1013,	0.3190	1	0.0001*
140	140	184	0.7609	63	159	0.3962	45.4511	0.2609,	0.4683	1	<0.001*

*= Statistically significant RA = restricted application of insecticide

Table 3b. Low infestation category – *Rhipicephalus* ticks

	RA			Control							
Days	**x**	**N**	**prop**	**x**	**N**	**prop**	χ^2	**95%CI**		**df**	**p-value**
0	27	203	0.1330	57	173	0.3295	19.6647	-0.2860,	-0.1069	1	<0.001*
28	48	182	0.7308	107	186	0.5753	35.3541	-0.4125,	-0.2104	1	<0.001*
56	104	194	0.5361	83	172	0.4826	0.842	-0.0544,	0.16148	1	0.359
84	112	174	0.6437	93	180	0.5167	5.3461	0.0194,	0.2346	1	0.021*
112	51	180	0.2833	75	154	0.4870	13.8014	-0.3124,	-0.0948	1	0.0002*
140	44	184	0.2391	95	159	0.5975	43.9706	-0.4622,	-0.2544	1	<0.001*

***= Statistically significant RA = restricted application of insecticide**

Table 3c: Medium infestation category – *Rhipicephalus* ticks

	RA			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	χ^2	**95%CI**		**df**	**p-value**
0	0	203	0.0000	6	173	0.0347	5.1164	-0.0673,	-0.0020	1	0.024*
28	1	182	0.0055	15	186	0.0806	10.7504	-0.1211,	-0.0291	1	0.001*
56	4	194	0.0206	70	172	0.4070	81.9897	-0.4679,	-0.3047	1	<0.001*
84	8	174	0.0460	69	180	0.3833	57.1951	-0.4205,	-0.2541	1	<0.001*
112	0	180	0.0000	1	154	0.0065	0.0061	-0.0252,	0.0122	1	0.938
140	0	184	0.0000	1	159	0.0063	0.0054	-0.0244,	0.0118	1	0.942

***= Statistically significant RA = restricted application of insecticide**

Table 3d. High infestation category – *Rhipicephalus* ticks

	RA			Control							
Days	**x**	**N**	**Prop**	**x**	**N**	**Prop**	χ^2	**95%CI**		**df**	**p-value**
0	1	203	0.0049	0	173	0	0	-0.0096,	0.0194	1	1
28	0	182	0	0	186	0	0	-0.020,	0.021	1	1
56	0	194	0	7	172	0.0407	6.0261	-0.0757,	-0.0056	1	0.014*
84	0	174	0	13	180	0.0722	10.9480	-0.1157,	-0.0287	1	0.001*
112	0	180	0	0	154	0	0	-0.024,	0.021	1	1
140	0	184	0	0	159	0	0	-0.024,	0.020	1	1

***= Statistically significant RA = restricted application of insecticide**

APPENDIX 11

Table 6.11a. Mean monthly haemoglobin concentration (g/dl) in the control and isometamidium chloride treated cattle.

Day	ISMM	Control	95%CI	d.f.	t-value	*p*-value
-42	10.67	10.92	-0.604 to 0.104	478	-1.39	0.166
-14	10.95	11.48	-0.879 to -0.181	379	-2.98	0.003*
0	11.29	11.69	-0.787 to -0.026	350	-2.1	0.036*
28	11.07	11.84	-1.139 to -0.408	345	-4.16	< 0.001*
56	11.57	12.06	-0.895 to -0.097	308	-2.44	0.015*
84	11.71	11.84	-0.515 to 0.248	344	-0.69	0.491
112	11.48	11.75	-0.643 to 0.118	299	-1.36	0.176
140	11.23	11.52	-0.740 to 0.164	296	-1.25	0.211

*** = statistically significant ISMM = isometamidium chloride**

Table 6.11b Mean monthly haemoglobin concentration (g/dl) in the control and pour-on treated cattle.

Day	Pour-on	Control	95% CI	d.f.	t-value	*p*-value
-42	10.64	10.92	-0.668 to 0.121	476	-1.36	0.174
-14	11.62	11.48	-0.229 to 0.515	354	0.75	0.451
0	11.7	11.69	-0.386 to 0.397	327	0.03	0.979
28	12.05	11.84	-0.173 to 0.601	329	1.09	0.277
56	12.26	12.06	0.202 to 0.593	325	0.97	0.334
84	12.02	11.84	-0.196 to 0.562	344	0.95	0.343
112	12.34	11.75	0.191to 0.988	304	2.91	0.004*
140	12.68	11.52	0.705 to 1.601	313	5.06	< 0.001*

*** = statistically significant**

Table 6.11c Mean monthly haemoglobin concentration (g/dl) in the control and RA treated cattle.

Day	Spray	Control	95% CI	d.f.	t-value	*p*-value
-42	10.51	10.92	-0.782 to -0.033	478	-2.14	0.033*
-14	11.4	11.48	-0.435 to 0.269	392	-0.47	0.642
0	11.72	11.69	-0.371 to 0.422	368	0.13	0.9
28	11.6	11.84	-0.604 to 0.117	357	-1.33	0.184
56	10.8	12.06	-1.669 to -0.865	359	-6.2	< 0.001*
84	11.04	11.84	-1.207 to -0.391	351	-3.85	< 0.001*
112	11.21	11.75	-0.919 to -0.149	331	-2.73	0.007*
140	11.59	11.52	-0.420 to 0.550	314	0.26	0.792

*** = statistically significant RA = restricted application**

APPENDIX 12

Table 12.1a. Proportion of animals in fat category.

	ISMM			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**d.f.**	***p*-value**
0	10	199	0.0502	11	173	0.063584	-0.0661, 0.0394	0.1093	1	0.741
28	5	169	0.0297	6	185	0.032432	-0.0418, 0.0361	0	1	1
56	13	144	0.0903	6	173	0.034682	-0.0049, 0.1161	3.3808	1	0.0660
84	13	166	0.0783	9	180	0.05	-0.0293, 0.0859	0.7358	1	0.391
112	13	151	0.0861	9	154	0.058442	-0.0370, 0.0923	0.5068	1	0.4765
140	1	141	0.0071	2	159	0.012579	-0.0332, 0.0222	0	1	1

ISMM = isometamidium chloride

Table 12.1b. Proportion of animals in medium category

	ISMM			Control						
Day	x	N	Prop	x	N	Prop	95% CI	χ^2	**d.f.**	***p*-value**
0	180	199	0.9045	159	173	0.9191	-0.0776, 0.0484	0.0958	1	0.7569
28	151	169	0.8935	178	185	0.9622	-0.1284, -0.0090	5.3425	1	0.0208*
56	129	144	0.8958	165	173	0.9538	-0.1232, 0.0073	3.1049	1	0.0786
84	150	166	0.9036	171	180	0.9500	-0.1072, 0.0144	2.1231	1	0.1451
112	136	151	0.9007	145	154	0.9416	-0.1079, 0.0261	1.24	1	0.2655
140	138	141	0.9787	157	159	0.9874	-0.0448, 0.0274	0.0184	1	0.8922

*** = difference is statistically significant ISMM = isometamidium chloride**

Table 12.1c. Proportion of animals in Lean category

	ISMM			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**d.f.**	***p*-value**
0	9	199	0.0452	3	173	0.0173	-0.0123, 0.0681	1.4984	1	0.2209
28	13	169	0.0769	1	185	0.0054	0.0243, 0.1187	10.0844	1	0.0015*
56	2	144	0.0139	1	173	0.0058	-0.0204, 0.0367	0.0256	1	0.873
84	3	166	0.0181	0	180	0.0000	-0.0080, 0.0441	1.5157	1	0.2183
112	2	151	0.0132	0	154	0.0000	-0.0115, 0.0380	0.5233	1	0.4694
140	2	141	0.0142	0	159	0.0000	-0.0120, 0.0404	0.6337	1	0.426

Table 12.2a: Proportion of animals in fat category

	Pour-on			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**d.f.**	***p*-value**
0	15	163	0.0920	11	173	0.0636	-0.0349, 0.0918	0.5942	1	0.4408
28	6	159	0.0377	6	185	0.0324	-0.0391, 0.0497	0	1	1.0000
56	13	166	0.0783	6	173	0.0347	-0.0114, 0.0987	2.2793	1	0.1311
84	11	166	0.0663	9	180	0.0500	-0.0390, 0.0715	0.174	1	0.6766
112	18	153	0.1176	9	154	0.0584	-0.0104, 0.1288,	2.6564	1	0.1031
140	5	158	0.0316	2	159	0.0126	-0.0196, 0.0577	0.5973	1	0.4396

*** = difference is statistically significant**

Table 12.2b: Proportion of animals in medium category

	Pour-on			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**d.f.**	***p*-Value**
0	140	163	0.8589	159	173	0.9191	-0.1333, 0.0129	2.518	1	0.1126
28	146	159	0.9182	178	185	0.9622	-0.1005, 0.0126	2.2639	1	0.1324
56	146	166	0.8795	165	173	0.9538	-0.1387, -0.0098	5.2209	1	0.0223*
84	155	166	0.9337	171	180	0.9500	-0.0715, 0.0390	0.174	1	0.6766
112	134	153	0.8758	145	154	0.9416	-0.1363, 0.0048	3.2481	1	0.0715
140	153	158	0.9684	157	159	0.9874	-0.0577, 0.0196	0.5973	1	0.4396

*** = difference is statistically significant**

Table 12.2c: Proportion of animals in lean category

	Pour-on			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**df**	***p*-Value**
0	8	163	0.0491	3	173	0.0173	-0.0127, 0.0761	1.7616	1	0.1844
28	7	159	0.0440	1	185	0.0054	-0.0008, 0.0780	4.0431	1	0.0444*
56	7	166	0.0422	1	173	0.0058	-0.0021, 0.0749	3.417	1	0.0645
84	0	166	0.0000	0	180	0.0000	-0.024, 0.023	0	1	1
112	1	153	0.0065	0	154	0.0000	-0.0127, 0.0258	0	1	0.9974
140	0	158	0.0000	0	159	0.0000	-0.024, 0.024	0	1	1

Table 12.3a: Proportion of animals in fat category

	Spray			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**d.f.**	**p-Value**
0	21	203	0.1034	11	173	0.0636	-0.0210, 0.1007	1.4287	1	0.2320
28	21	182	0.1154	6	185	0.0324	0.0245, 0.1414	8.0853	1	0.0045*
56	20	194	0.1031	6	173	0.0347	0.0122, 0.1246	5.5041	1	0.0190*
84	18	192	0.0938	9	180	0.0500	-0.0137, 0.1012	2.0318	1	0.1540
112	24	180	0.1333	9	154	0.0584	0.0069, 0.1429	4.4206	1	0.0355*
140	7	158	0.0443	2	159	0.0126	-0.0110, 0.0745	1.8558	1	0.1731

*** = difference is statistically significant**

Table 12.3b: Proportion of animals in medium category

	Spray			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**d.f.**	**p-Value**
0	172	203	0.8473	159	173	0.9191	-0.1412, -0.0024	3.9123	1	0.0479
28	158	182	0.8681	178	185	0.9622	-0.1558, -0.0322	9.3086	1	0.0023*
56	171	194	0.8814	165	173	0.9538	-0.1330, -0.0116	5.2841	1	0.0215*
84	171	192	0.8906	171	180	0.9500	-0.1192, 0.0004	3.6530	1	0.0560
112	155	180	0.8611	145	154	0.9416	-0.1491, -0.0118	5.0275	1	0.0250*
140	151	158	0.9557	157	159	0.9874	-0.0745, 0.0110	1.8558	1	0.1731

Table 12.3c : Proportion of animals in lean category

	Spray			Control						
Day	**x**	**N**	**Prop**	**x**	**N**	**Prop**	**95% CI**	**χ^2**	**df**	**p-Value**
0	10	203	0.0493	3	173	0.0173	-0.0090, 0.0728	1.975	1	0.1599
28	3	182	0.0165	1	185	0.0054	-0.0157, 0.0378	0.2696	1	0.6036
56	3	194	0.0155	1	173	0.0058	-0.0165, 0.0359	0.1508	1	0.6978
84	3	192	0.0156	0	180	0.0000	-0.0073, 0.0385	1.2185	1	0.2697
112	1	180	0.0056	0	154	0.0000	-0.0108, 0.0220	0	1	1.0000
140	0	158	0.0000	0	159	0.0000	-0.024, 0.024	0	1	1